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REDACTED COPY

April 12, 2005

VIA HAND DELIVERY

Ms. Kelly White
Office of Pesticide Programs
Environmental Protection Agency
1801 Bell Street, Room 266A
Arlington, VA 22202

Re: **Chloropicrin UCSD Chloropicrin Human Sensory Irritation Study**

Dear Ms. White:

I am writing on behalf of the Chloropicrin Manufacturers Task Force in response to your letter of March 3, 2005, requesting additional information on the Human Sensory Irritation Testing for Chloropicrin, (MRID No. 46443801). In your letter you asked for (1) additional information regarding the institutional review board and its review, (2) additional information regarding testing procedures and apparatus and, (3) additional data analysis. This letter responds solely to your questions regarding the institutional review board. The response to the other questions will be sent separately.

Please note that the attachments include proprietary commercial information, personal financial information and other information that is exempted from disclosure under 40 C.F.R. Part 2. I have included two copies of the attachments. One copy is for internal EPA use and the other is a pubic copy with the relevant pages redacted.

Question 1 - Are the Institutional Review Board and the Human Subjects Committee the same?

Answer: Yes. In addition, it is important to note that, the IRB/Human Subjects Committee meets the criteria set forth in 40 C.F.R. §§ 26.107 (IRB Membership), 26.108 (IRB function and operations), 26.109 (IRB Research Review) and 26.111 (Criteria for IRB approval of research). Attachment 1 is a letter from Mamie Gonzalez of UCSD regarding the Human Subject Protection Program which explains that all IRB committee operations are compliant with the Common Rule (40 C.F.R. Part 26 and 45 C.F.R. Part 46).

Question 2: Please provide documentation of all reviews by either group including the initial review, the review of protocol revisions and the reviews of the research in progress.

Answer: Enclosed are the following documents:

- The Study Director's, Dr. William Cain, June 2000 submission to the IRB, including the proposed protocol (Attachment 2);
- The IRB's responses to the 2000 submission (Attachment 3);
- Dr. Cain's February 2001 submission to the IRB[1] and all related correspondence with the IRB in 2001, including comments and approvals. (Attachment 4);
- the IRB's 2002 renewal approval including Dr. Cain's request (Attachment 5);
- the IRB's 2003 renewal approval including Dr. Cain's submission to the IRB (Attachment 6); and,
- the IRB's 2004 renewal approval including Dr. Cain's request (Attachment 7).

Questions 3: Please provide all versions of the protocol.

Answer: All versions of the protocol reviewed by the IRB are included in Attachments 2 through 7. Attachments 2 through 7 also contain the IRB's comments on the protocols and revisions made to the protocol in light of those comments.

Question 4: Please provide copies of all materials used in participant recruitment and in the informed consent process.

Answer: A copy of the invitation to participate and flyers used for participant recruitment are in Attachment 8. The informed consent forms are included in the various protocols, which were provided in response to Question 2.

[1] The IRB treated the February 2001 submission as a new submission.

CMTF has worked with Dr. Cain to assemble this information and believes that it responds to your questions. If you have any additional questions, please contact me at the above number.

Sincerely,

Sara Beth Watson

cc: William S. Cain
John Butala
CMTF w/o attachments

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

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MAMIE GONZALEZ
DIRECTOR
HUMAN RESEARCH PROTECTIONS PROGRAM

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To whom it may concern:

The four Institutional Review Boards of the University of California, San Diego (UCSD) are duly constituted to conform to all federal regulation requirements of the U.S. Food and Drug Administration (FDA) and the Office for Human Research Protections (OHRP) that govern research involving human subjects. They are composed of at least five members, with varying backgrounds to promote complete and adequate review of research activities commonly conducted at this institution. The Institutional Review Boards include at least one member whose primary concerns are in the scientific areas and at least one member whose concerns are in nonscientific areas. Each also includes one member who is not otherwise affiliated with the institution and who is not part of the immediate family of a person who is affiliated with the institution. IRB members are of both genders, and fulfill federal requirements for diversity. No member of the Institutional Review Board may participate in the review, or vote on any project in which the member has a conflicting interest, except to provide information requested by the IRB. A list of the Institutional Review Board members, by profession, and gender is on file with DHHS.

The UCSD Human Research Protections Program (HRPP) has written procedures for initial and continuing review, prepares written minutes of convened meetings, and retains records pertaining to the review and approval process. IRB committee operations are compliant with requirements defined in 21 CFR (Code of Federal Regulations) Parts 50 and 56, and 45 CFR 46, as well as ICH (International Conference on Harmonization) guidance relating to Good Clinical Practice. UCSD has an approved Federal Wide Assurance of Compliance on file with the Department of Health and Human Services (DHHS). The identification number of this Assurance is FWA00004495. This Assurance is effective for the period of April 4, 2003 through April 4, 2006.

This Federal Wide Assurance of Compliance applies to all research conducted under the aegis of the University of California, San Diego, which includes research conducted at all UCSD medical facilities, including the UCSD Medical Center, the Thornton Hospital, and the San Diego Veterans Affairs Healthcare System.

Questions regarding the program and its federal assurances may be directed to (858) 455-5050.

M. Gonzalez

Mamie Gonzalez

APPLICATION TO COMMITTEE ON INVESTIGATIONS INVOLVING HUMAN SUBJECTS

PRINCIPAL INVESTIGATOR William S. Cain

William S. Cain _____
Signature of P.I. Date

Title Prof. Dept Surgery Mail Code 0957
Salaried? Yes ✓ No ___ Phone 858-622-5831
Contact Person W.S. Cain Mail Code 0957
Contact Phone 858-622-5831 Fax 858-9917

OTHER INVESTIGATORS A. Talwayzki, Terence Davidson, Thomas Bruff, & Roland Schmidt

PROJECT TITLE

Human Sensory Irritation Testing for Chloropicrin

NEW ✓ If not new, please provide:

1. Previous Project No. _____
2. Previous Expiration Date _____

Be sure to include a **SUMMARY OF EXPERIENCE** with this project to date (under Section #5) and an **EXPLANATION** of any differences between this submission and the previously approved project.

INDICATE WHETHER PROJECT WILL INVOLVE:

	YES	NO
Subjects under 18	___	✓
Fetuses	___	✓
Pregnant Women	✓	___
Women of child-bearing-potential	✓	___
Mentally Retarded/Disabled	___	✓
University Patients/Facilities	✓	___
Surgical ICU (University facilities)	___	✓
General Clinical Research Center facilities	___	✓
VAMC Patients	___	✓
VAMC Facilities	___	✓
Gene therapy trial	___	✓

FUNDING SUPPORT	YES	NO
Is project currently supported by:		
Existing extramural funds?	___	___
Agency/Agencies _____		
Grant/Contract # _____		
Gift funds?	___	___
Department funds?	___	___
VMRF?	___	___
Unfunded study?	___	___
Solicited extramural funds not yet received?	✓	___
Agency/UCSD Proposal # Chloropicrin Mfg Task Group		
Proposal Title: Human Sensory Irritation Testing for Chloropicrin		
Is this a Drug/Device Study?		No

Company/Entity: _____
UCSD Clinical Trial Agreement # _____
NOTE: Should funding entity change, another 730U form is required. Please notify Conflict of Interest at 534-6465, mail code 0992.

Project Number _____ Date Received _____
(leave blank)

Signature of Department Chairperson 6/15/00
Date

Sponsor (If PI is a student) Date

RADIOISOTOPES/X-RAY MACHINE USE

1) Will any radioisotopes be used? Yes___ No ✓ **If yes:** Under which Radiosotope Use Authorization (RUA) will the work be performed? UCSD#_____. VA#_____
   a. Which isotopes? _____
   b. Routine Nuclear Medicine Procedure? Yes___ No___
      Procedure Name: _____
   c. Non-routine procedure? Yes___ No___ **If yes:**
      Procedure Type _____
      IND, NDA or IDE # _____
2) Will any "x-ray" procedures be used? Yes___ No___
   a. Routine clinical x-ray procedures? Yes___ No___
      Procedure Name: _____
   b. Non-Routine procedures? Yes___ No___
      Procedure Type: IDE#

DRUGS

Name all DRUGS & DOSES to be used.

None

INVESTIGATIONAL NEW DRUGS & DEVICES

New drugs (including drugs used in a new manner or form) and devices usually require clearance for investigational use from the U.S. Food and Drug Administration.

Note all such "test articles" and their IND/IDE number here: _____, _____, _____

THIS IS A COVER SHEET ONLY

Submit **20 * COMPLETE** copies of the application **(please staple)** including this page. Also, include three **(3)** copies of the **Master Protocol** and three **(3)** copies of the **Investigators Brochure** to:
LUCILLE PEARSON: Human Subjects Committee 0052

***For ALL cancer related studies, submit:* 20 COMPLETE** copies plus three **(3)** copies of the Master protocol. **Send an additional 10 copies and 1 copy of the Master Protocol to the Cancer Protocol Committee, ATTN: PRC Office 0698.**

*For minimum risk protocols, submit three **(3)** copies (See page on Expedited Review).

Call x44520 for additional information if needed.

Expected TOTAL ACCRUAL At This Site: 47.00 N=

Title of Project: Human Sensory Irritation Testing for Chloropicrin

Principal Investigator: William S. Cain, Ph.D., Professor of Surgery (Otolaryngology)

Co-Investigators: Alfredo Jalowayski, Ph.D., Research Specialist, Depts. Of Pediatrics and Surgery (Otolaryngology), Terence M. Davidson, M.D., Professor of Surgery (Otolaryngology-HNS), Thomas Bruff, M.D., M.P.H., Assistant Clinical Professor of Medicine, Center for Occupational & Environmental Medicine, and Roland Schmidt, Ph.D., Postdoctoral Fellow, Dept. of Surgery (Otolaryngology)

1. Facilities: The work will be performed in the Chemosensory Perception Laboratory at the La Jolla Village Professional Center.

2. Duration: One to two years.

3. Specific Aims: There are three phases of work, each with a specific aim, as follows:

Aim 1: To establish the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the sites.

Aim 2: To establish sensitivity for ambient exposures where all channels for detection are available and, within this framework, to establish whether sensitivity varies over time for exposures that range from a few seconds to 30 minutes. Of particular interest with respect to time-course is whether undetectable concentrations become detectable over time.

Aim 3: To establish whether just-irritating ambient exposures of one-half hour per day over four days lead to evidence of inflammation in the eyes or airways.

Aims 1 and 2 pertain to all persons, those exposed adventitiously from environmental releases of chloropicrin and those exposed occupationally. Aim 3 pertains to persons exposed occupationally and more likely to have repetitive exposure.

4. Background and Significance

Chloropicrin is a colorless liquid with a sharp, penetrating odor. The material is used primarily to fumigate fields in order to control soil-borne fungi, plant diseases, and nematodes. Pre-planting fumigation with chloropicrin makes it possible for plants such as strawberries to achieve exceptional root-growth unaffected by soil-pests and disease. Because of the sharpness of its vapors, chloropicrin has also been added to other, odorless fumigants, such as sulfuryl flouride and methyl bromide as a warning agent. The

same property of sharpness has led to use of the material as an agent to test the vapor attenuating properties of activated carbon and the fit of gas masks.

Human beings come into contact with chloropicrin principally on the job in agriculture. The American Conference of Governmental Industrial Hygienists (ACGIH) initially set the threshold limit value (TLV) at 1 ppm (time-weighted average, TWA) in 1957 and reduced it to 0.1 ppm in 1959, where it remains today. In the documentation, ACGIH states: "A TLV-TWA of 0.1 ppm is recommended for repeated exposure to chloropicrin to prevent eye irritation and the potential for pulmonary changes" (p. 299). OSHA in the U.S. and its regulatory counterparts in many industrial countries also use a TWA of 0.1 ppm as the permissible exposure limit.

The repellent properties of chloropicrin at low levels include blepharospasm, tearing, and pungency. As Krieger (1996) noted in an exposure and risk assessment: "This protective reflex response is an important homeostatic mechanism by which exposure is reduced and the sensitive human pulmonary system is spared adverse effects resulting from higher levels of chloropicrin. ... It is a protective reflex response that occurs when chloropicrin contacts the trigeminal nerves of the moist nasal airways. Tearing (lachrymation) and painful stinging eyes results from temporary disturbances of the eye that are completely reversible and occur at concentrations of 0.15 ppm to 0.3 ppm chloropicrin. This undeniable reflex response warns persons to move to uncontaminated environments before toxic exposures occur." (Pp. 10-11).

Krieger noted further that the mandated use of chloropicrin to warn of the presence of odorless fumigants "gives clear evidence of regulatory recognition of the importance and usefulness of the warning properties of low levels of chloropicrin" (p. 12). To illustrate, U. S. EPA PR NOTICE 84-5 describes the language to be used for methyl bromide that contains chloropicrin: "This product contains chloropicrin as a warning odorant. Chloropicrin may be irritating to the upper respiratory tract, and even at low levels can cause painful irritation to the eyes, producing tearing. If these symptoms occur, leave the fumigation area immediately." (U. S. EPA, 1984; p. A-1).

Table 2 from Krieger (1996) shows human responses to airborne chloropicrin at various levels and at exposures from instantaneous to chronic to the extent that these have been studied. In the main, the effects derive from direct contact between chloropicrin and tissue at the portal of entry. The nausea and vomiting that may occur from high levels of exposure are thought to occur because of swallowing of the irritating material that has dissolved into saliva.

Testing in laboratory animals by inhalation or other routes of exposure has indicated that chloropicrin in nontoxic exposures poses no hazard to pregnancy or to the developing fetus, nor does it impair reproductive functioning. It has been found to be noncarcinogenic in the species tested (two strains of rats, two strains of mice, Beagle dogs). There is no evidence that it will bioaccumulate in mammalian cells.

Table 2. Human Responses to Airborne Chloropicrin Exposures

Exposures	Conc. x Time	Response	Characteristics
Workplace or Off-Site	Less than TLV-TWA 0.1 ppm or less	None No adverse effects(eye):NOAEL	None
Instantaneous Contact	0.15 to 0.3 ppm x secs	*Common Chemical Sense Threshold*	*Tolerable with very slight or no irritating sensation*
		Reflex tearing and reflex coughing	① Concentration dependent
		Protects lower respiratory tract	② Response ceases when exposure removed
			③ Response non-cumulative
			④ Primary irritant, little or no systemic effect
	TLV-TWA: 0.1 ppm or less		
Acute	Greater than TLV-TWA of 0.1 ppm; seconds-minutes	Irritation	*Slightly irritating, some stinging or burning of eye, nose, throat*
	0.15-0.3 ppm; 3-30 secs	Lacrimation	
	0.3-0.37 ppm; 2-2.5 seconds	Eye irritation; LOAEL	
	0.9 ppm	Odor threshold	
	1.5 ppm; few seconds		⑤ Secondary airway irritant; lacrimation, nausea, vomiting
	4 ppm; few seconds	Incapacitation	*Intolerable; rapidly incapacitating*
	15 ppm; few seconds	Pulmonary Toxicity	Upper respiratory tract irritation; tissue injury; edema
Chronic	0.1 ppm	No Effect	None

The present investigation concerns specification of the lowest concentrations of chloropicrin detectable by human beings. The data regarding when chloropicrin will be felt in the eyes or the nose have come from anecdotal reports, rather than from controlled studies. The investigation will address two situations: 1) the environmental case, where a person typically unconnected with the application of chloropicrin (e.g., a resident or passerby) is exposed, and 2) the occupational case, where exposures may occur episodically over some days. In that case, the work goes beyond specification of mere detection and into the consequences of relatively brief (30 min) repeated exposures at a low level of irritation. Consequences of interest include low-level inflammatory reactions of the eyes and upper airways. No animal study can supplant the human tests for precision or relevance.

This research plan was devised in response to a request for proposals from the Chloropicrin Manufacturers Task Force. The protocol was, however, created by the investigators at UCSD.

5. Progress

We have not previously studied this chemical and there appear to be no modern controlled psychophysical studies of it.

6. Research Design and Methods

Phase 1. Measurement of Sensitivity to Momentary Exposures

Objective: To establish in 50 screened subjects (25 males and 25 females) the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the individual sites. Exposures will represent initial perception via the nose and eyes. Relationships of interest will comprise psychometric (concentration-response) functions for olfactory sensitivity, sensitivity to nasal pungency, and sensitivity of the eyes. The highest points on such functions represent consistent detection of the material, not more.

Test Material: Chloropicrin (CCl_3NO_2; CAS #76-06-2) will be the test material throughout the investigation. It is liquid at room temperature (b.p. 112°C). It has a vapor pressure similar to that of water (18.3 mm Hg @ 20°C; 24 mm Hg @ 25°C), has relatively low solubility in water (1.6 g/L @ 25°C), and is miscible in most organic solvents. Chloropicrin will be received in the laboratory in 2-ml quantities in order to insure that in handling of samples no large amounts can spill.

Apparatus: Concentrations of chloropicrin will be presented in phase 1 by the vapor delivery device (VDD) shown in Fig. 1. It will be placed inside an environmental chamber where ventilation rate will equal 20 air changes per hour. A concentration series will be set up at the beginning of a day and samples taken for calibration at a point shown to be

at steady state. Calibrations will need to lie within 20% of nominal values for testing to proceed. Calibration will be repeated at appropriate intervals during a day of testing.

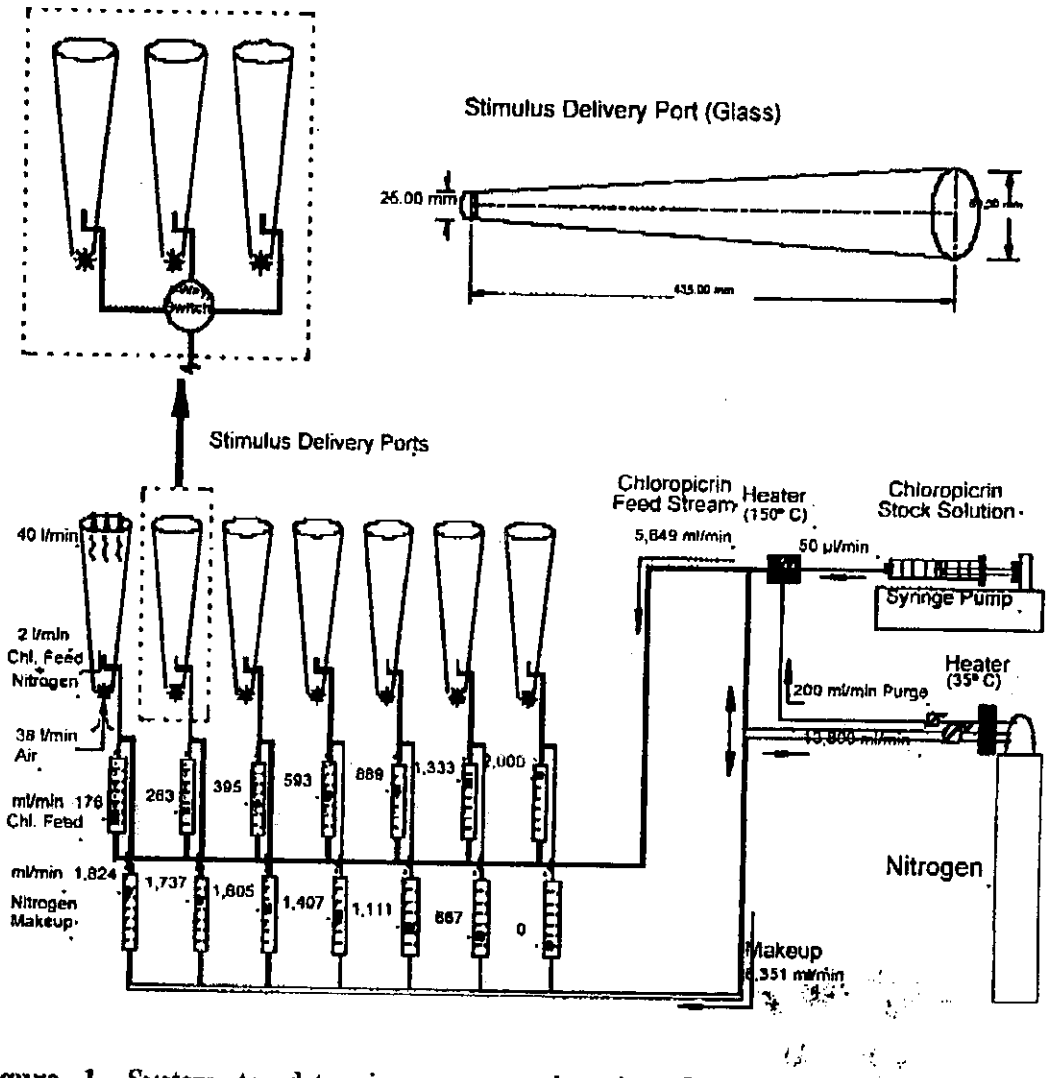


Figure 1. System to determine sensory detection for momentary exposures to chloropicrin vapor. The system will entail splitting a feed stream of chloropicrin-containing nitrogen into seven lines and diluting each with nitrogen and air to achieve the concentration of interest at the end of glass conical delivery ports.

Procedure: After they have passed screening, subjects will be scheduled for tests with chloropicrin. The subjects will need to agree to participate in four sessions of approximately 5 hours each over a period of weeks. On a day of testing of chloropicrin, six subjects will report to the laboratory approximately 20-30 min before the beginning of testing and will fill out forms to indicate their nasal and ocular health on that day. They will have been told not to eat or drink within the previous 30 min. On the first day of

testing for a subject, he/she will receive orientation regarding how to sample from the delivery cones. He will need to learn how his progress through a series will be signaled with tones to tell him how long to stay in contact with a cone and when to move on to the next cone and the next station.

On any given day, the VDD will be set for an odor series or an irritation series. (We should note that if subjects detect chloropicrin from its feel or irritation at concentrations below its odor threshold, then testing detection via odor will be pointless.) All subjects within the group of six will be making the same judgments, i.e., of odor, of ocular irritation, or of nasal pungency. During testing, subjects will follow one another through the exposures in round-robin fashion.

A given subject will move in progression through the various stations of the VDD from low to higher concentrations on a single pass and will make many passes in a session, up to 30. After a pass, he will queue up at the end of the group and wait to begin his next round.

At each station of the VDD, the subject will encounter three cones and will need to choose one as containing the test material (three-alternative forced choice). It will take approximately 30 sec to sample the three (five sec per cone, with five sec in-between). After indicating a choice, the subject will move to the next higher level. There will be a 30-sec time-out between the sampling of different levels. For testing of the nose, subjects will wear swimmers' goggles to protect their eyes from the vapor and, for the testing of the eyes, the subjects will wear nose clips. (Pilot work will determine whether subjects are more sensitive to the odor or to the nasal pungency of chloropicrin. Depending on the answer, minor modifications in procedure may be necessary.) A subject will have at least a five-min break between the end of a pass and the beginning of another, an interval adequate for recovery from any sensory adaptation.

Data analysis: From a subject's various passes through the series in a day, it will be possible to erect a psychometric function. Over his three days of testing with chloropicrin, he will produce a function for odor, a function for nasal pungency, and a function for ocular irritation. A function will be accepted into the data set by a criterion of goodness of fit to a theoretical function to be determined from pilot testing. The most likely choice will be a log Gaussian ogive.

Failure of a function to reach a criterion goodness of fit can indicate lapses of attention on the part of a subject or inability to perform the task. This will be diagnosed within a day because the data will be entered into a spreadsheet for analysis on the same day as a test. This will serve an important role in quality control. A subject who performs unreliably may be given another opportunity, but will be dropped if his function fails to show consistency in a second session.

Psychometric functions will be analyzed for their slopes and intercepts, with slope indicative of intra-subject variability and intercept indicative of absolute sensitivity, and ultimately of inter-subject variability. Both the entire functions and certain key parameters, such as the concentrations that lead to 50% correct detection above chance, will be analyzed by ANOVA to inquire about associations of sensitivity with sex, nasal health (as explained below), and age.

Phase 2. Specification of the Sensory Response to Exposures Up to 30 Minutes

Objective: To establish in 25 males and 25 females sensitivity for ambient exposures where all channels for detection are available and, within this framework, to establish whether sensitivity varies over time for exposures that range from a few seconds to 30 minutes.

Apparatus: The laboratory is equipped with four chambers of dimensions 4' x 8' x 8' (Fig. 2). Each can hold four seated occupants. The chambers have a single-pass ventilation system that delivers air from a perimeter base-board and exhausts it at the ceiling. Ventilation rate can be varied over a wide range. Testing will take place at normal temperature (22°C) and humidity (RH 40-50%).

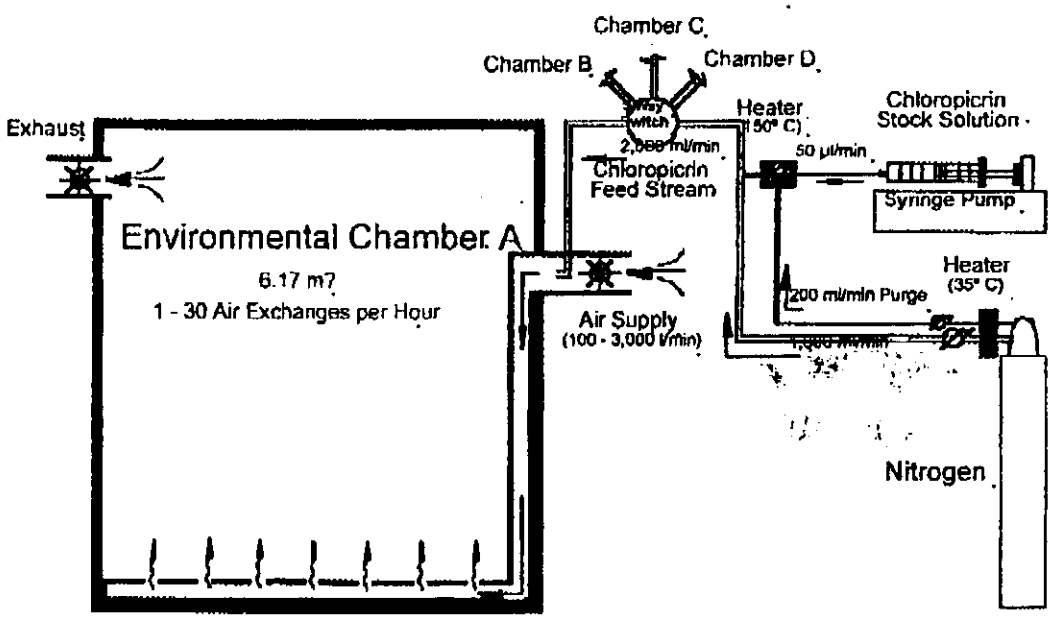


Figure 2. System to determine detection of chloropicrin in four environmental chambers over periods ranging from 5 sec to 30 min. This system will require concentrations to be varied from one segment in time to another.

Levels of chloropicrin will be achieved as indicated in Fig. 2. Four or five levels will be studied. These will be monitored by personal sampling pumps placed in the

breathing zone of occupants. Samples taken with an adsorbent will be desorbed in the gas chromatograph for analysis.

Procedure: In brief exposures, subjects will be exposed to the air in the chamber for 5 sec at a time and make a judgment regarding whether or not the chamber appears to contain test material. This will be done with a rating of confidence: "Is test material present? *Yes* or *No*. Rate confidence from 0 = no confidence, to 5 = high confidence." In addition, subjects will be asked to note through which site(s) they perceived the test material.

For all testing in the chambers, the subject will wear a respirator until told to remove it. The reason for the device is that opening and closing the door to a chamber to let in subjects will disturb its atmosphere and subjects will need to be in the environment for a time for the atmosphere to achieve steady state. (Subjects will be fitted with appropriate respirators and taught how to keep them snug. We will test goodness of fit of the respirators in a session before any exposure. It should be borne in mind that the levels will be relatively difficult to detect even without a respirator.) Four subjects facing away from one another can occupy a chamber at once. All four chambers can have four occupants in these circumstances and after a trial has been completed the occupants can move into another chamber and repeat the process. The chamber with the test material can be varied after every fourth exposure and, on that occasion, the concentration changed. This will occur over a series of concentrations until detection is consistent. (Pilot work will lead to the appropriate range and steps.) The information about which chamber contains the test material and the level of the test material will be unknown to the person who deals with the subjects, i.e., the study will be double blind. We will build a switching device that will feed the chambers appropriate levels of vapor and control flow as necessary via a feedback loop.

For the brief duration, subjects will not need to track their sensations while in the chamber, but for the long duration, they will rate irritation periodically throughout the exposure. Nevertheless, the judgment of confidence regarding whether the chamber had the test material will still be the judgment of most interest for it can be compared to whether or not test material was present.

Each subject will be tested at four to five concentrations twice. We anticipate that this can be accomplished in one session per subject for the short duration and two to three for the long duration.

Data Analysis: The outcome of this testing will be the subjects' ratings of confidence in the cases when the test material is present vs. when it is absent. The data can be analyzed via ANOVA for its interval properties, i.e., actual ratings, in order to determine the concentration that can just be discriminated from blank air. The data can also be analyzed for its nominal properties, i.e., correctness vs. incorrectness, to construct receiver operating characteristic (ROC) curves to show level of discrimination. The confidence ratings will be analyzed to test the hypothesis that time alters sensitivity.

Phase 3: Daily Exposures and Signs of Inflammation

Objective: To establish in 10 males and 10 females whether just-irritating ambient exposures of one-half hour per day over four days lead to evidence of inflammation in the eyes or upper airways. The level of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways.

Apparatus: Exposures will take place in the chambers. Concentration will be controlled as indicated above in phase 2. A concentration detectable approximately 75 % of the time will be used.

Procedure: Exposures in the chambers will last one-half hour on four successive days. In one span of four days, subjects will have exposure to chloropicrin at a just-irritating level, to be determined from previous runs (phase 2), and, in another span, those same subjects will have exposure to just air on four successive days. Half the subjects will have exposure to chloropicrin first and half exposure to air first. At least two weeks will separate these periods. Subjects will rate relative levels of irritation during exposures, but the focal concern will be changes that imply development of inflammation throughout a span.

The sessions will run over six working days beginning on a Friday. On that day, baseline measures will be taken. With the exception of a Rhinoprobe sample, the baseline measures will be taken again the following Monday, just before the first exposure of the four that will occur that week. The following will be performed:

1) a Rhinoprobe scraping will be taken (Friday only) from the inferior turbinate to establish the number and composition of cells in the mucosal layer,

2) office spirometry will be performed to establish forced vital capacity (FVC) and forced expiratory volume at 1 sec (FEV_1),

3) nasal resistance will be measured by anterior rhinomanometry,

4) rate and composition of nasal secretion will be assessed via placement of a 7-mm diam. sponge on the septum for 30 sec,

5) exhaled nitrogen oxide (NO) will be measured via the mouth, to indicate NO generated in the lungs, and via the nose, to indicate nasal-sinus emission of NO,

6) a photograph of one eye will be taken for subsequent judgment of redness,

7) tear fluid will be taken on a sponge for 2-min for analysis of composition of ocular secretion, and

8) an impression sample will be taken on 3-mm diam. membrane filters in four locations of the conjunctiva inside the lower eye-lid.

During a session, the following will be performed:

1) respiratory rate will be monitored remotely via Respibands, and

2) blink rate will be counted remotely via videography.

Thirty min after a session, the following will be performed:

1) office spirometry, as before the session,

2) nasal resistance, as before the session,

3) nasal secretion will be collected, as before the session,

4) exhaled NO will be measured, as before the session,

5) mucociliary clearance will be measured via the time to taste a saccharin crystal placed on the lower turbinate in the nasal cavity,

6) a photograph of the eye will be taken, as before the session,

7) tear fluid will be collected, as before the session, and

8) impression sample will be taken from inside the lower eye-lid, as before the session.

Approximately 24 hr after the last session, the subject will return and the following will be performed:

1) a second Rhinoprobe sample, and

2) the measurements taken 30 min after the sessions.

Data Analysis: For interpretation of the study, we need to make two sets of distinctions: 1) some variables will be measured to monitor the safety of the subjects and some to establish the responses of substantive interest, and 2) among the variables of substantive interest, some reflect normal physiological responses and some likely pathophysiological responses.

With respect to safety:

Pulmonary function will be compared between after and before exposure to examine whether a subject has experienced bronchoconstriction from the exposure. A

reduction of FEV_1 of 15 % will be reason to remove a subject from further exposures at the level that caused the constriction.

If concentration of NO from the lungs increases by 20%, this will give reason to remove the subject.

With respect to substantive interest:

Nasal resistance will be compared after vs before exposure as an aspect of dose of chloropicrin vapor. A blocked nasal passage means lower dose than a patent passage. An increase in resistance is a normal response to inhalation of an irritating vapor. Resistance can also provide a means to interpret concentration of NO from the nasal cavity.

Respiratory rate will serve as an aspect of dose.

Blink rate will serve as a remote index of whether subjects can feel vapor in their eyes.

Secretions collected on the sponge placed on the septum will be analyzed first for total mass. Total amount of secretion should increase in an environment where irritation is experienced.

The secretions collected in the nose will be analyzed for concentrations of albumin, soluble intercellular adhesion molecule-1 (sICAM), and IL-8, as indicators of inflammation.

The secretions collected from the eye will also be analyzed for concentrations of albumin, soluble intercellular adhesion molecule-1 (sICAM) and IL-8, as indicators of inflammation.

The pictures of the eye will be compared between after and before.

The impression sample taken from the lower eye-lid will be analyzed for the presence of cells associated with inflammation. This analysis will give the definitive measure of inflammation.

The Rhinoprobe sample will similarly be analyzed for the presence of cells associated with inflammation.

7. Human Subjects

Subjects (18-35 years of age) will be screened for participation in a two-part process that will include a phone interview and laboratory visit, with testing. In the phone interview, subjects will be excluded for any of the following:

1. History of occupational exposure to chloropicrin.

2. Chronic cough, history of chronic pulmonary disease, chronic sinusitis, nasal polyposis, active allergic rhinitis, or asthma.

3. History of acute or chronic cardiovascular, liver, or kidney disease.

4. Acute illness within the previous month.

5. Investigational exposures to pollutants within the previous two weeks.

6. History or evidence of chemical sensitivity.

7. History of ocular abnormalities, other than a need for glasses.

8. History of alcohol or drug abuse.

9. Smoking within the previous year.

10. Daily use of medication, excluding birth control pills.

11. Absence of sense of smell.

In an initial visit to the laboratory, subjects will: a) give a medical history, b) take a brief test of olfaction to assure that they can smell everyday objects normally, c) go through screening of the nasal and ocular mucosae, and d) have office spirometry testing.

Screening for disorders of the upper airways will entail clinical examination, assessment of nasal resistance, and cytology from material collected in a Rhinoprobe scraping from the inferior turbinate. Subjects will be excluded for signs of clinically-relevant inflammation of the upper airways, for indications of viral or bacterial infectious rhinitis, and for excessive nasal resistance. In the cytological exam, the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells will be taken as evidence for respiratory viral infection, whereas large numbers of neutrophils (3+ or 4+) with intracellular bacteria will be taken as evidence of bacterial infection. Large numbers of eosinophils or basophilic cells (3+ or 4+) will also be taken as indicative of inflammation. Such levels generally accompany a clinically-relevant degree of allergic rhinitis, a condition that should also be evident from the medical history.

The office spirometry testing will conform to the recommendations of the American Thoracic Society. Data to be acquired from this test include the following: FVC, the total volume of gas exhaled after a full inspiration, and FEV_1, the gas volume exhaled in one second by a forced expiration from a full inspiration. The data will allow calculation of the ratio FEV_1/FVC. Failure of pulmonary function to lie at or above 75% of predicted FEV_1 or FVC will result in exclusion.

Ocular screening will entail inspection under slit-lamp illumination for surface abnormalities (scarring, ulceration) or abnormal redness and cytology from an impression-sample taken from the conjunctival membrane inside the lower eyelid. The lower lid is sampled with a 3-mm diam. filter placed at the end of a rod. The rod weighs exactly 60 g and suspension of the rod within a short outer cylinder at the moment of contact insures that the 60 g will be exerted on the lid. Dr. Alfredo Jalowayski, who has developed our ocular procedures, will perform the slit-lamp evaluation.

Persons with abnormalities of the ocular surface will be excluded, as will persons with significantly elevated levels of PMNs (neutrophils, eosinophils, and basophilic cells) in the conjunctival membrane. Persons with small elevations above normal will not be excluded. We will hold open the option of stratifying on the cytological variable in order to discover whether evidence of inflammation accounts for any part of psychophysical sensitivity, i.e., for individual differences in sensitivity, a matter not yet known.

Use of contact lenses will not preclude participation. Subjects who use such lenses will be tested with them in place.

8. Informed Consent

Subjects will be recruited from the general population. Formal consent will be sought just prior to screening, typically by the person who will administer the tests. The consent form will contain the relevant facts regarding the reason for the study, the task at hand, any possible adverse effects, and the opportunity to withdraw at any time without penalty. Consent will be written and the subject will receive a copy of the form along with the subject's bill of rights.

9. Therapeutic Alternatives

Not a therapeutic study.

10. Potential Risks

Screening: The screening procedures of direct visualization of the nasal and ocular mucosae and measurement of nasal resistance pose no realistic risks that we can anticipate. Sampling from the nasal mucosa has been performed routinely by Dr. Jalowayski in the Nasal Dysfunction Clinic for many years and entails minimal risk. The impression cytological sampling from the lower lid, also entails minimal risk.

Testing: The risks associated with testing entail sensory irritation of the upper airways or eyes and remotely in these circumstances asthma. We say "remotely" because the exposures will be to perithreshold concentrations with respect to irritation.

11. Risk Management

Monitoring: Subjects will be monitored visually during testing. Testing will stop if the subject finds the test situation uncomfortable and asks to stop. We will instruct subjects to report any respiratory symptoms immediately.

Actions to be taken in an adverse event occurs:

Upper Airway or Ocular Discomfort: Irritation of the nose or eyes should subside immediately after exposure. Should it last longer than expected, the subject will be instructed regarding how to irrigate the affected area with saline solution. An eye irrigation system exists within the laboratory. Furthermore, over-the-counter preparations for such purposes will be available on-site for the subject to use before leaving the lab and to take home. The subject will be informed that if any such irritation or discomfort fails to show progressive improvement during the ensuing hours, then she should page Dr. Terence Davidson who will oversee medical management of the symptoms.

Lower airway reactions: Should exposure cause shortness of breath from apparent bronchoconstriction, then an ACLS-certified staff member will initiate an asthma-management protocol. (This person, to be hired, will have training appropriate to obtain ACLS certification. Candidates will be nurses, respiratory therapists, and EMTs.) An emergency kit to treat acute asthma will include: a) a Ventolin inhaler (albuterol USP inhalation aerosol), b) an AeroChamber to be used with the metered dose inhaler, c) a nebulizer and oxygen source, d) a pulse oxymeter, and e) an epinephrine auto-injector (EpiPen) to deliver a 0.3 mg intramuscular dose of epinephrine (1:1000), and d) apparatus for oxygen therapy.

The protocol for management of acute asthma will be the following: a) if symptoms occur, the subject will be offered treatment with Ventolin (one puff held in the lungs for 10 sec., followed by a second puff 30 sec later), b) the pulse oxymeter will be attached to a digit in order to monitor oxygen saturation, c) if the subject seems unable to inhale from the inhaler adequately, then the nebulizer will be used, d) Dr. Thomas Bruff will be paged and informed of the event, and he will decide how the subject should be managed, e) if the subject obtains relief within 15 min. and Dr. Bruff gives no instructions to the contrary, the subject will be asked to remain in the lab for the next half-hour or until he no longer feels short of breath; he will be called at intervals over the next 8-24 hr to inquire about any late reaction, e) if the subject fails to obtain relief, he will be transported to the emergency room at Thornton Hospital, less than 2 miles from the lab. If the subject shows signs of anaphylactic shock, he can be given an injection of epinephrine from the EpiPen and administered humidified oxygen.

Regarding privacy, the experimental results will be available only to the investigators.

12. Potential Benefits

There will be no benefits to individual subjects.

This research is motivated by the need to develop a definitive set of data relevant to environmental and occupational exposures to chloropicrin. Since the chemical has very low systemic toxicity compared to its rather strong irritating properties, it is irritation that will dictate permissible exposure levels. (The irritation response, as we have noted, largely protects against systemic exposure.) In so far as the present research makes it possible to set such levels with a greater margin of protection, then its potential benefit to occupational and public health is considerable.

We can mention that approximately 50% of permissible exposure levels, e.g., the threshold limit values of the American Conference of Governmental Industrial Hygienists, are based explicitly upon sensory irritation and yet there have been almost no controlled studies of such. We seem to be entering a new era when companies and trade groups that exercise product stewardship over commodity chemicals seek quantitative data on human sensory irritation. As product stewards, these entities are often asked to advise users of their products about hazardous properties. Indeed, they must do so in Material Safety Data Sheets. If the data to advise about the properties do not exist, or if they are poor, then it is the obligation of the product steward to obtain it. This matter goes beyond merely setting permissible exposure levels into more intrinsic understanding of the perceptual and physiological effects of the chemicals. We see these developments as salutary.

14. Risk-Benefit Ratio: Minor risk, no individual benefits, but benefits to society as indicated just above.

15. Expense to subject: None anticipated.

16. Privileges/Certifications and Licenses: Dr. Davidson has privileges at UCSD Medical Center. He indicated to the Human Subjects Program in 1999 that sampling from mucosal tissue, the only procedure where we touch the subject, does not require direct medical supervision. Dr. Jalowayski, who has worked with Dr. Davidson in the Nasal Dysfunction Clinic for more than 15 years, developed the sampling and performs it routinely in the clinic. Dr. Jalowayski will do the sampling in this project.

Dr. Thomas Bruff, board certified in internal medicine and in occupational medicine, is attending staff at UCSD Medical Center. He is ACLS-certified.

17. Bibliography:

American Conference of Governmental Industrial Hygienists (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th ed. Cincinnati: ACGIH.

Kharitinov, S. A., Rajakulasingam, K., O'Connor, B., et al. (1997). Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. Journal of Allergy and Clinical Immunology, 99 (1), 58-64.

Krieger, R. I. (1996). An assessment of implied worker exposure and risk associated with chloropicrin loading, application, and field tarping activities following application, and implied exposure and risk for off-field concentrations resulting from soil fumigation. Registration document submitted to CAL-EPA Department of Pesticide Regulation, October 1996.

Silkoff, P. E., Robbins, R. A., Gaston, B., Lundberg, J. O. N., & Townley, R. G. (2000). Endogenous nitric oxide in allergic airway disease. Journal of Allergy and Clinical Immunology, 105 (3), 438-448.

U. S. Environmental Protection Agency (1984). Notice to Manufacturers, Formulators, Producers and Registrants of Fumigant Products. PR Notice-84-5.

Wagenmann, M., Baroody, F. M., Desrosiers, M., Hubbard, W. S., Ford, S., Lichtenstein, L. M., & Naclerio, R. M. (1996). Unilateral nasal allergen challenge leads to bilateral release of prostaglandin D_2. Clinical and Experimental Allergy, 26, 371-378.

18. Industry Studies: Supported by the Chloropicrin Manufacturer's Task Group. The Group approached us about the need for the information and we designed the study. The UCSD Office of Contracts and Grants Administration (Pamela Tiffany) has negotiated a contract with the Group. Activation of the contract awaits approval of the human subjects committee. The University will retain all patent rights and intellectual rights to the results and is free to publish the work. The PI has no personal agreements (e.g., consultation, confidentiality) with the Chloropicrin Manufacturer's Task Group.

19. Other Funding: None.

20. Cancer Studies: Not applicable.

21. Biological Materials Transfer Agreement: No materials to be transported.

22. Investigational Drug Fact Sheet: No investigational drugs to be used.

23. Conflict of Interest: Form submitted here.

24. Nursing Staff: No impact.

25. Information Service: Will be completed.

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor

Phases 1 & 2

William S. Cain, Ph.D., and associates are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Group, on the perception of irritation via the nose and eyes and the perception of odor from chloropicrin. The chemical is used commonly as a fumigant in agriculture. The research is intended to specify the thresholds where perception of the material occurs. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

If you agree to participate, you will be one of approximately 25 male and 25 female subjects between ages 18 and 35.

Your involvement will entail approximately 5 sessions of up to 5 hours over a period of a few weeks and scheduled at mutual convenience.

In order to participate in this work, you should already have answered no regarding the following questions:

1. History of occupational exposure to chloropicrin.
2. Chronic cough, history of lung problems or nasal problems.
3. History of cardiovascular, liver, or kidney disease.
4. Acute illness within the previous month.
5. Investigational exposures to other pollutants or contaminants within the previous two weeks.
6. History of chemical sensitivity.
7. History of ocular abnormalities, other than a need for glasses.
8. History of alcohol or drug abuse.
9. Smoking within the previous year.
10. Daily use of medication, excluding birth control pills.

11. Absence of the sense of smell.

Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history anda brief test of the sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), customary to the medical clinic, will be administered by Dr. Alfredo Jalowayski. If you don't pass screening, you will be excused and paid for your time.

In sessions performed after screening, your task will be to make judgments about whether you experience irritation or odor in brief exposures of your eyes or nose to just air or to very low concentrations of chloropicrin vapor.

You will receive $10 per hour for your time in screening and in testing.

We don't expect any direct benefits to you of participation in this research. There should, however, be benefits to public health from development of standards for exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. We expect the discomfort to be very minor and short lasting. The discomfort we can anticipate is some irritation in the nose, throat, and eyes. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you should experience an adverse effect during testing, we will provide the necessary care on site. If that is insufficient, we will escort you to emergency care.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those injuries. The sponsor, Chloropicrin Manufacturers Task Group Corporation, will pay all reasonable medical and hospital costs required for diagnosis and treatment of any injury caused by the research.. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

Research records will be kept confidential to the extent provided by law.

You may keep a copy of this consent document and "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor

Phase 3

William S. Cain, Ph.D., and associates are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Group, on sensory irritation via the nose and eyes from the substance chloropicrin. The chemical is used commonly as a fumigant in agriculture. The research is intended to establish whether exposures to chloropicrin at levels that are just-irritating will lead to signs of inflammation in the nose and eyes. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

If you agree to participate, you will be one of approximately 10 male and 10 female subjects between ages 18 and 35.

Your involvement will entail approximately 13 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

In order to participate in this work, you should already have answered no regarding the following questions:

1. History of occupational exposure to chloropicrin.
2. Chronic cough, history of lung problems or nasal problems.
3. History of cardiovascular, liver, or kidney disease.
4. Acute illness within the previous month.
5. Investigational exposures to other pollutants or contaminants within the previous two weeks.
6. History of chemical sensitivity.
7. History of ocular abnormalities, other than a need for glasses.
8. History of alcohol or drug abuse.
9. Smoking within the previous year.
10. Daily use of medication, excluding birth control pills.

11. Absence of the sense of smell.

Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history anda brief test of the sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), customary to the medical clinic, will be administered by Dr. Alfredo Jalowayski. If you don't pass screening, you will be excused and paid for your time.

In sessions performed after screening, we will perform various tests of how your nose and eyes respond to 30-minute exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in two six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all six days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your nose and mouth.

You will receive $20 for your time in screening and $250 for your time in the remainder of the tests ($10/hour).

We don't expect any direct benefits to you of participation in this research. There should, however, be benefits to public health from development of standards for exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. We expect the discomfort to be very minor and short lasting. The discomfort we can anticipate is some irritation in the nose, throat, and eyes. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet

our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you should experience an adverse effect during testing, we will provide the necessary care on site. If that is insufficient, we will escort you to emergency care.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those injuries. The sponsor, Chloropicrin Manufacturers Task Group Corporation, will pay all reasonable medical and hospital costs required for diagnosis and treatment of any injury caused by the research.. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

Research records will be kept confidential to the extent provided by law.

You may keep a copy of this consent document and "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

000765
PENDING

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN SUBJECTS COMMITTEE

Date: July 25, 2000

To: Dr. William S. Cain Mailcode: 0957

Re: Project #000765
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

The Committee voted to approve this study pending receipt of:

1. Clarification as to how the data will be used once it is obtained.
2. A copy of the research proposal and expected outcomes in response to a request for proposal from the CMTF as indicated on p.4 of the IRB application.
3. An expanded discussion/description of the various tasks. These need to be more specific.
4. Clarification regarding payment to subjects. If subjects are paid $10.00 per hour, and there are 13 sessions of up to 3 hours each, this is more than $250.00.
5. Clarification whether the sponsor will have access to the research records. If yes, this should be discussed in the consent.
6. Clarification in the consent as to how strong the irritation may become for subjects.
7. A copy of the recruitment flyer.
8. A revised consent that follows the UCSD IRB format.

Please send your reply along with two copies of the revised consent (one clean copy and one copy **underlining or bolding** the changes) to the Human Subjects Program Office, 0052. Final approval will be forwarded just as soon as we can determine that your responses are satisfactory.

Sincerely,

Lucille Pearson/bz

Lucille Pearson, Director
Human Subjects Program
0052 X44520

000765
30 Day

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN SUBJECTS COMMITTEE

Date: October 3, 2000

To: Dr. William S. Cain Mailcode: 0957

Re: Project #000765
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

This letter is to remind you that approval for the above-referenced project has not yet been granted. In order for approval to be granted, the conditions and requirements set forth in the Committee's letter of July 25, (copy enclosed) must be satisfied within 30 days of today's letter.

If you have any questions about this policy or the contents of our original letter, please call me at x44520.

Sincerely,

Lucille Pearson/sc

Lucille Pearson, Director
Human Subjects Program
0052 X44520

000765
10 DAY

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN SUBJECTS COMMITTEE

Date: November 30, 2000

To: Dr. William S. Cain Mailcode: 0957

Re: Project #000765
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

Since we have received no response to our letter of October 3, 2000, regarding the above-referenced project, I assume that you wish to have it withdrawn from further consideration.

Please let me know if this assumption is not **correct** within 10 days of the date of this letter.

Sincerely,

Lucille Pearson/smc

Lucille Pearson, Director
Human Subjects Program
0052 X44520

APPLICATION TO COMMITTEE ON INVESTIG[illegible]

HSC Proj Nr: 010201 Mtg: 03/01/2001
"Human Sensory Irritation Testing for Chloropicrin"
P.I.: William S. Cain, Ph.D.
Primary: Crawford Second: Wallace

PRINCIPAL INVESTIGATOR William S. Cain

WCain 1/16/01
Signature of P.I. Date

Title Prof IR Dept Surgery Mail Code 0957
Salaried? Yes ✓ No ___ Phone 858-622-5831
Contact Person W.S. CAIN Mail Code 0957
Contact Phone 858-622-5831 Fax 458-9417

OTHER INVESTIGATORS Alfredo Talavera, M.D., Terence Davidson, M.D., Roland Schmitt, M.D., Thomas Bruff, M.D., M.P.H.

PROJECT TITLE
Human Sensory Irritation Testing for Chloropicrin

NEW ✓ If not new, please provide:

1. Previous Project No. ___ FEB 08 2001
2. Previous Expiration Date ___

Be sure to include a **SUMMARY OF EXPERIENCE** with this project to date (under Section #5) and an **EXPLANATION** of any differences between this submission and the previously approved project.

INDICATE WHETHER PROJECT WILL INVOLVE:

	YES	NO
Subjects under 18	—	✓
Fetuses	—	✓
Pregnant Women	—	✓
Women of child-bearing-potential	✓	—
Mentally Retarded/Disabled	—	✓
University Patients/Facilities	✓	—
Surgical ICU (University facilities)	—	✓
General Clinical Research Center facilities	—	✓
VAMC Patients	—	✓
VAMC Facilities	—	✓
Gene therapy trial	—	✓

FUNDING SUPPORT	YES	NO
Is project currently supported by:		
Existing extramural funds?	—	—
Agency/Agencies ___		
Grant/Contract # ___		
Gift funds?	—	—
Department funds?	—	—
VMRF?	—	—
Unfunded study?	—	—
Solicited extramural funds not yet received?	✓	—
Agency/UCSD Proposal # Not yet assigned		
Proposal Title: Human Sensory Irritation Testing for Chloropicrin		
Is this a Drug/Device Study	—	✓

Company/Entity: ___
UCSD Clinical Trial Agreement # ___

NOTE: **Should funding entity change, another 730U form is required. Please notify Conflict of Interest at 534-6465, mail code 0992.**

___ Date

Sponsor (If PI is a student) Date

RADIOISOTOPES/X-RAY MACHINE USE

1) Will any radioisotopes be used? Yes___ No___ **If yes:** Under which Radioisotope Use Authorization (RUA) will the work be performed? UCSD#_____, VA#_____
   a. Which isotopes? _____
   b. Routine Nuclear Medicine Procedure? Yes___ No___
      Procedure Name: _____
   c. Non-routine procedure? Yes___ No___ **If yes:**
      Procedure Type _____
      IND, NDA or IDE #. _____
2) Will any "x-ray" procedures be used? Yes___ No___
   a. Routine clinical x-ray procedures? Yes___ No___
      Procedure Name: _____
   b. Non-Routine procedures? Yes___ No___
      Procedure Type: IDE#

DRUGS

Name all DRUGS & DOSES to be used.

None

INVESTIGATIONAL NEW DRUGS & DEVICES

New drugs (including drugs used in a new manner or form) and devices usually require clearance for investigational use from the U.S. Food and Drug Administration.

Note all such "test articles" and their IND/IDE number here: _____, _____, _____

THIS IS A COVER SHEET ONLY

Submit **20 * COMPLETE** copies of the application **(please staple)** including this page. Also, include three **(3)** copies of the **Master Protocol** and three (3) copies of the **Investigators Brochure** to:
LUCILLE PEARSON: **Human Subjects Committee 0052**

***For ALL cancer related studies, submit:* 20 COMPLETE** copies plus three **(3)** copies of the Master protocol. **Send an additional 10 copies and 1 copy of the Master Protocol to the Cancer Protocol Committee, ATTN: PRC Office 0698.**

*For minimum risk protocols, submit three (3) copies (See page on Expedited Review).

Call x44520 for additional information if needed.

Expected TOTAL ACCRUAL At This Site ≈150

Title of Project: Human Sensory Irritation Testing for Chloropicrin

Principal Investigator: William S. Cain, Ph.D., Professor of Surgery (Otolaryngology)

Co-Investigators: Alfredo Jalowayski, Ph.D., Research Specialist, Depts. of Pediatrics and Surgery (Otolaryngology), Terence M. Davidson, M.D., Professor of Surgery (Otolaryngology-HNS), Thomas Bruff, M.D., M.P.H., Assistant Clinical Professor of Medicine, Center for Occupational & Environmental Medicine, and Roland Schmidt, Ph.D., Postgraduate Researcher, Dept. of Surgery (Otolaryngology)

1. Facilities: The work will be performed in the Chemosensory Perception Laboratory at the La Jolla Village Professional Center.

2. Duration: One to two years.

3. Specific Aims: There are three phases of work, each with a specific aim, as follows:

Aim 1: To establish the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the sites.

Aim 2: To establish sensitivity for ambient exposures where all channels for detection are available and, within this framework, to establish whether sensitivity varies over time for exposures that range from a few seconds to 30 minutes. Of particular interest with respect to time-course is whether undetectable concentrations become detectable over time.

Aim 3: To establish whether mildly irritating ambient exposures of one-half hour per day over four days lead to a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue.

Aims 1 and 2 pertain to all persons, those exposed adventitiously from environmental releases of chloropicrin and those exposed occupationally. Aim 3 pertains primarily to persons exposed occupationally and more likely to have repetitive exposure. Aim 3 can also apply to residential exposure because off-gassing may occur for several days from a single field. If there are multiple fields in an area, residents could conceivably be exposed intermittently for a longer time.

4. Background and Significance

Chloropicrin is a colorless liquid with a sharp, penetrating odor. The material is used primarily to fumigate fields in order to control soil-borne fungi, plant diseases, and nematodes. Pre-planting soil fumigation with chloropicrin makes it possible for plants such as strawberries to achieve exceptional root-growth unaffected by soil-pests and

disease. Because of the sharpness of its vapors, chloropicrin has also been added to other, odorless fumigants, such as sulfuryl flouride and methyl bromide as a warning agent. The same property of sharpness has led to use of the material as an agent to test the vapor attenuating properties of activated carbon and the fit of personal protective respiratory devices.

The mammalian acute and chronic toxicology of chloropicrin, including that following inhalation, is well described and current. Testing in laboratory animals by inhalation or other routes of exposure has indicated that chloropicrin in nonsystemically toxic exposures poses no hazard to pregnancy or to the developing fetus, nor does it impair reproductive functioning. It has been found to be noncarcinogenic following lifetime inhalation exposure to rats and mice and in chronic feeding studies to beagle dogs. A chronic oral (gavage) study in rats produced a single animal with a stomach papilloma in the test group receiving 10mg/kg/day, the highest dose tested. This papilloma is considered to be spontaneous in origin. There is no evidence that chloropicrin will bioaccumulate in mammalian cells.

Human beings come into contact with chloropicrin principally on the job in agriculture or, to a lesser extent, in wood preservation. The American Conference of Governmental Industrial Hygienists (ACGIH) initially set the threshold limit value (TLV) at 1 ppm (time-weighted average, TWA) in 1957 and reduced it to 0.1 ppm in 1959, where it remains today. In the documentation, ACGIH states: "A TLV-TWA of 0.1 ppm is recommended for repeated exposure to chloropicrin to prevent eye irritation and the potential for pulmonary changes" (p. 299). OSHA in the U.S. and its regulatory counterparts in many industrial countries also use a TWA of 0.1 ppm as the permissible exposure limit.

The repellent properties of chloropicrin at low levels include reflex blepharospasm, tearing, and pungency that can effectively function as warning properties. As Krieger (1996) noted in an exposure and risk assessment: "This protective reflex response is an important homeostatic mechanism by which exposure is reduced and the sensitive human pulmonary system is spared adverse effects resulting from higher levels of chloropicrin. ...It is a protective reflex response that occurs when chloropicrin contacts the trigeminal nerves of the moist nasal airways. Tearing (lachrymation) and painful stinging eyes results from temporary disturbances of the eye that are completely reversible and occur at concentrations of 0.15 ppm to 0.3 ppm chloropicrin. This undeniable reflex response warns persons to move to uncontaminated environments before toxic exposures occur." (Pp. 10-11).

Krieger noted further that the mandated use of chloropicrin to warn of the presence of odorless fumigants "gives clear evidence of regulatory recognition of the importance and usefulness of the warning properties of low levels of chloropicrin" (p. 12). To illustrate, U. S. EPA PR Notice 84-5 describes the language to be used for methyl bromide that contains chloropicrin: "This product contains chloropicrin as a warning odorant. Chloropicrin may be irritating to the upper respiratory tract, and even at low levels can cause painful irritation to the eyes, producing tearing. If these symptoms occur, leave the fumigation area immediately." (U. S. EPA, 1984; p. A-1).

Table 1, from Krieger (1996), shows human responses to airborne chloropicrin at various levels and at exposures from instantaneous to chronic to the extent that these have been studied. In the main, the effects derive from direct contact between chloropicrin and tissue at the portal of entry. The nausea and vomiting that may occur from high levels of exposure are thought to occur because of swallowing of the irritating material that has dissolved into saliva.

As detailed below, human responses to airborne chloropicrin are known mainly through anecdotal reports or from studies and other observations collected many decades ago. These experiences indicate that exposure to airborne chloropicrin concentrations of about 0.15ppm to 0.3 ppm can cause tearing and eye irritation that is reversible upon cessation of exposure.

TABLE 1. Human Responses to Airborne Chloropicrin Exposures[1]

Exposures	Conc. x Time	Response	Characteristics
Workplace or Off-Site	Less than TLV-TWA 0.1 ppm or less	None No Adverse effects (eye): NOAEL	None
Instantaneous Contact	0.15 to 0.3 ppm x secs	*Common* Chemical *Sense Threshold*	Tolerable *with very slight or no irritating sensation*
		Reflex tearing and reflex coughing	① Concentration dependent
			②Response ceases when exposure removed
			③Response non-cumulative
			④Primary irritant, little or no systemic effect
	TLV-TWA: 0.1 ppm or less		
Acute	Greater than TLV-TWA of 0.1 ppm; seconds-minutes	Irritation	*Slightly irritating, some stinging or burning of eye, nose, throat*
	0.15-0.3 ppm; 3.30 secs.	Lacrimation	
	0.3-0.37 ppm; 2-2.5 seconds	Eye irritation; LOAEL	
	0.9 ppm	Odor threshold	
	1.5 ppm; few seconds		⑤ Secondary airway irritant; lacrimation, nausea, vomiting
	4 ppm; few seconds	Pulmonary Toxicity	Upper respiratory tract irritation; tissue injury; edema
Chronic	0.1 ppm	No Effect	None

[1]To the best of our knowledge, no controlled human studies of chloropicrin exposure have been completed. Each of these values in this table is anecdotal or derives from a source for which analytical verification of chloropicrin concentrations and standardized evaluation of subject response does dot exist. The present protocol describes a laboratory study that incorporates proper and comprehensive control of variables as well as appropriate analytical and psychophysical response measurement technology to assure valid results having the greatest degree of scientific certainty..

The present investigation concerns specification of the lowest concentrations of chloropicrin detectable by human beings. The investigation will address two situations: 1) the environmental case, where a person typically unconnected with the application of chloropicrin (e.g., a resident or passerby) is exposed, and 2) the occupational case, where exposures may occur episodically over some days. In that case, the work goes beyond specification of mere detection and into the consequences of relatively brief (30 min) repeated exposures at a low level of irritation. Possible consequences of interest include low-level inflammatory reactions of the eyes and upper airways. No animal study can supplant the human tests for precision or relevance. To conduct these studies, human volunteers are to be exposed to chloropicrin vapor and evaluated for ocular irritation, nasal irritation, odor perception (of chloropicrin) and for indications of pulmonary irritation in a laboratory setting in which chloropicrin vapor concentrations and sensory responses can be readily and precisely monitored. Goals of the research are to establish thresholds for these responses, to begin to understand the magnitude of human response variability, if any, to chloropicrin exposure and to improve understanding of differences between animal and human responses to chloropicrin.

The results of these studies will provide an appropriate framework to learn whether odor or sensory irritation which signals low-level exposure to chloropicrin provides an adequate mechanism to prevent occurrence of adverse effects seen in high-dose animal studies. This work also will allow evaluation of important human attributes of the responses to low-level chloropicrin; individual variability in responses; differences in sensitivity as a function of modality of response (ocular stimulation vs, nasal stimulation); and repeated vs. single exposure responses that may be important manifestations of the sensory response to chloropicrin.

Together, the information from these studies will be useful not only in human hazard assessment but also for the promulgation of exposure standards for workers and the general population.

This research plan was devised in response to a request for proposals from the Chloropicrin Manufacturers Task Force. The protocol was, however, created by the investigators at UCSD.

5. Progress

We have not previously studied this chemical and there appear to be no modern controlled psychophysical studies of it.

6. Research Design and Methods

Phase 1. Measurement of Sensitivity to Momentary Exposures

Objective: To establish in 50 screened subjects (25 males and 25 females) the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the individual sites. Exposures will represent initial perception via the nose and eyes. Relationships of interest will

comprise psychometric (concentration-response) functions for olfactory sensitivity, sensitivity to nasal pungency, and sensitivity of the eyes. The highest points on such functions represent consistent detection of the material, not more.

Test Material: Chloropicrin (CCl_3NO_2; CAS #76-06-2) will be the test material throughout the investigation. It is liquid at room temperature (b.p. 112°C). It has a vapor pressure similar to that of water (18.3 mm Hg @ 20°C; 24 mm Hg @ 25°C), has relatively low solubility in water (1.6 g/L @ 25°C), and is miscible in most organic solvents. Chloropicrin will be received in the laboratory in 2-ml quantities in order to insure that in handling of samples no large amounts can spill.

Table 4
Guide for Grading Nasal Cytograms

Quantitative Analysis	Semi-quantitative Analysis	Grade
Epithelial Cells		
N/A	Normal morphology	N
N/A	Abnormal morphology	A
N/A	Ciliocytophthoria	CCP
Eosinophils, neutrophils		
0*	None	0
0.1-1.0*	Occasional cells	0.5+
1.1-5.0*	Few scattered cells or small clumps	1+
5.1-15.0*	Moderate number of cells and larger clumps	2+
15.1-20.0*	Larger clumps of cells which do not cover the entire field	3+
>20*	Large clumps of cells covering the entire field	4+
Basophilic cells		
0*	None	0
0.1-0.3*	Occasional cells	0.5+
0.4-1.0*	Few scattered cells	1+
1.1-3.0*	Moderate number of cells	2+
3.1-6.0*	Many cells easily seen	3+
>6.0*	Large number of cells, as many as 25 per high power field	4+
Bacteria**		
N/A	None seen	0
N/A	Occasional clump	1+
N/A	Moderate number	2+
N/A	Many easily seen	3+
N/A	Large numbers covering the entire field	4+
Goblet cells***		
0	None	0
1-24%	Occasional to few cells	1+
25-49%	Moderate number	2+
50-74%	Many easily seen	3+
75-100%	Large number, may cover entire field	4+

* Mean of cells per 10 high power fields (x1000)
** Note presence of intracellular bacteria
*** Ratio of goblet cells to epithelial cells, expressed as percentage

Figure 1. System to determine sensory detection for momentary exposures to chloropicrin vapor. The system will entail splitting a feed stream of chloropicrin-containing nitrogen into seven lines and diluting each with nitrogen and air to achieve the concentration of interest at the end of glass conical delivery ports.

Apparatus: Concentrations of chloropicrin will be presented in Phase 1 by the vapor delivery device (VDD) shown in Fig. 1. It will be placed inside an environmental

chamber where ventilation rate will equal 20 air changes per hour. A concentration series will be set up at the beginning of a day and samples taken for calibration at a point shown to be at steady state. The maximum concentration in the series will lie in the vicinity of 0.1 to 0.3 ppm and adjacent steps will differ by a factor of approximately 1.25. Measurement of concentration will be performed via gas chromatography with an electron capture detector (ECD). Calibrations will need to lie within 20% of nominal values for testing to proceed. Calibration will be repeated at appropriate intervals during a day of testing.

Figure 2. Showing three stations of the vapor delivery device. Station #1 delivers the highest test concentration, station #2, the next higher, and so on. In testing, subjects start at the weakest concentration, station #8, and work progressively up to station #1. At each station, the cone with stimulus material, i.e, the active cone, can vary. The inactive cones deliver just background flow. Subjects are blinded to which cones are active.

Procedure: After they have passed screening, subjects will be scheduled for tests with chloropicrin. The subjects will need to agree to participate in four sessions of approximately 5 hours each over a period of weeks. On a day of testing of chloropicrin, six subjects will report to the laboratory approximately 20-30 min before the beginning of testing and will fill out forms to indicate their nasal and ocular health on that day. They will have been told not to eat or drink within the previous 30 min. On the first day of testing for a subject, he/she will receive orientation regarding how to sample from the delivery cones (Fig. 2). He will need to learn how his progress through a series will be signaled with tones to tell him how long to stay in contact with a cone and when to move on to the next cone and the next station.

On any given day, the VDD will be set for an odor series or an irritation series. (We should note that if subjects detect chloropicrin from its feel or irritation at concentrations below its odor threshold, then testing detection via odor will be pointless.)

All subjects within the group of six will be making the same judgments, i.e., of odor, of ocular irritation, or of nasal pungency. During testing, subjects will follow one another through the exposures in round-robin fashion.

A given subject will move in progression through the various stations (#8 through #1) of the VDD from low to higher concentrations on a single pass and will make many passes in a session, up to 30. After a pass, he/she will queue up at the end of the group and wait to begin his next round.

At each station of the VDD, the subject will encounter three cones and will need to choose one as containing the test material (three-alternative forced choice). It will take approximately 30 sec to sample the three (five sec per cone, with five sec in-between). After indicating a choice, the subject will move to the next higher level. There will be a 30-sec time-out between the sampling of different levels. For testing of the nose, subjects will wear swimmers' goggles to protect their eyes from the vapor and, for the testing of the eyes, the subjects will wear nose clips. (Pilot work will determine whether subjects are more sensitive to the odor or to the nasal pungency of chloropicrin. Depending on the answer, minor modifications in procedure may be necessary.) A subject will have at least a five-min break between the end of a pass (finishing station #1) and the beginning of another (starting again at station #8), an interval adequate for recovery from any sensory adaptation.

Data analysis: From a subject's various passes through the series of stations in a day, it will be possible to erect a psychometric function. Over his three days of testing with chloropicrin, he/she will produce a function for odor, a function for nasal pungency, and a function for ocular irritation. A function will be accepted into the data set by a criterion of goodness of fit to a theoretical function to be determined from pilot testing. The most likely choice will be a log-Gaussian ogive.

Failure of a function to reach a criterion goodness of fit can indicate lapses of attention on the part of a subject or inability to perform the task. This will be diagnosed within a day because the data will be entered into a spreadsheet for analysis on the same day as a test. This will serve an important role in quality control. A subject who performs unreliably may be given another opportunity, but will be dropped if his/her function fails to show consistency in a second session.

Psychometric functions will be analyzed for their slopes and intercepts, with slope indicative of intra-subject variability and intercept indicative of absolute sensitivity, and ultimately of inter-subject variability. Both the entire functions and certain key parameters, such as the concentrations that lead to 50% correct detection above chance, will be analyzed by ANOVA to inquire about associations of sensitivity with sex, nasal health (as explained below), and age.

Phase 2. Specification of the Sensory Response to Exposures Up to 30 Minutes

Objective: To establish in 50 screened subjects (25 males and 25 females) sensitivity for ambient exposures to chloropicrin where all channels for detection are available and, within this framework, to establish whether sensitivity varies over time for exposures that range from a few seconds to 30 minutes.

Apparatus: The laboratory is equipped with four chambers of dimensions 4' x 8' x 8' (Figs. 3 and 4). Each can hold four seated occupants. The chambers have a single-pass ventilation system that delivers air from a perimeter base-board and exhausts it at the ceiling. Ventilation rate can be varied over a wide range. Testing will take place at normal temperature (22°C) and humidity (RH 40-50%).

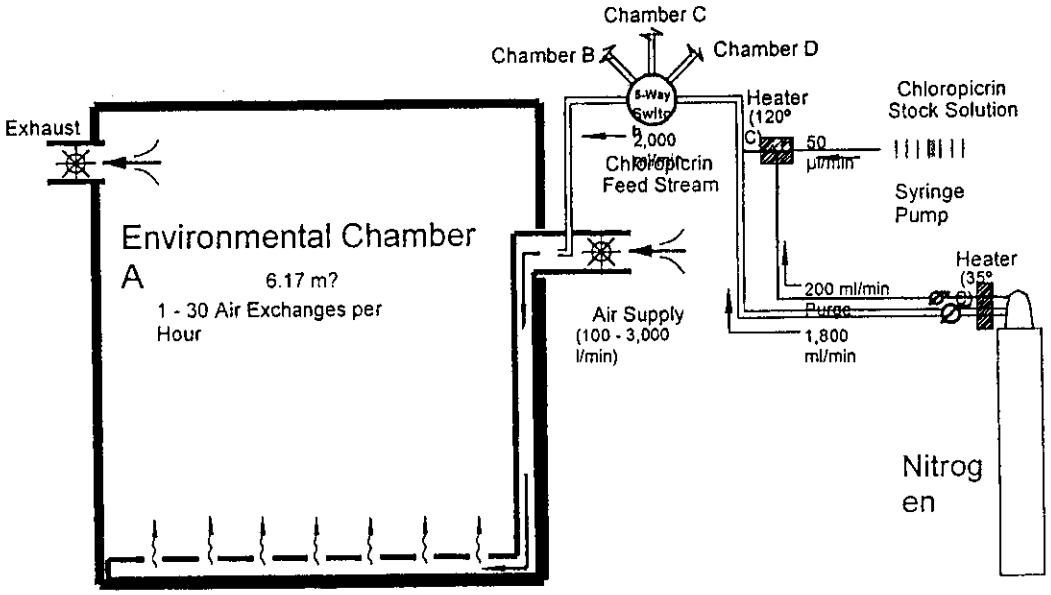


Figure 3. System to determine detection of chloropicrin in four environmental chambers over periods ranging from 5 sec to 30 min. This system will require concentrations to be varied from one segment in time to another.

Levels of chloropicrin will be achieved as indicated in Fig. 3. Four or five levels will be studied. Assuming that the levels under study exceed the lowest level of detection of the ECD, concentration in the chambers will be measured by direct injection of vapor-samples into the chromatograph (note sampling station in Fig. 4). If the levels do not exceed the lowest level of detection of the ECD, concentration will be monitored by personal sampling pumps placed in the breathing zone of occupants. Samples taken with an adsorbent will be desorbed in the gas chromatograph for analysis.

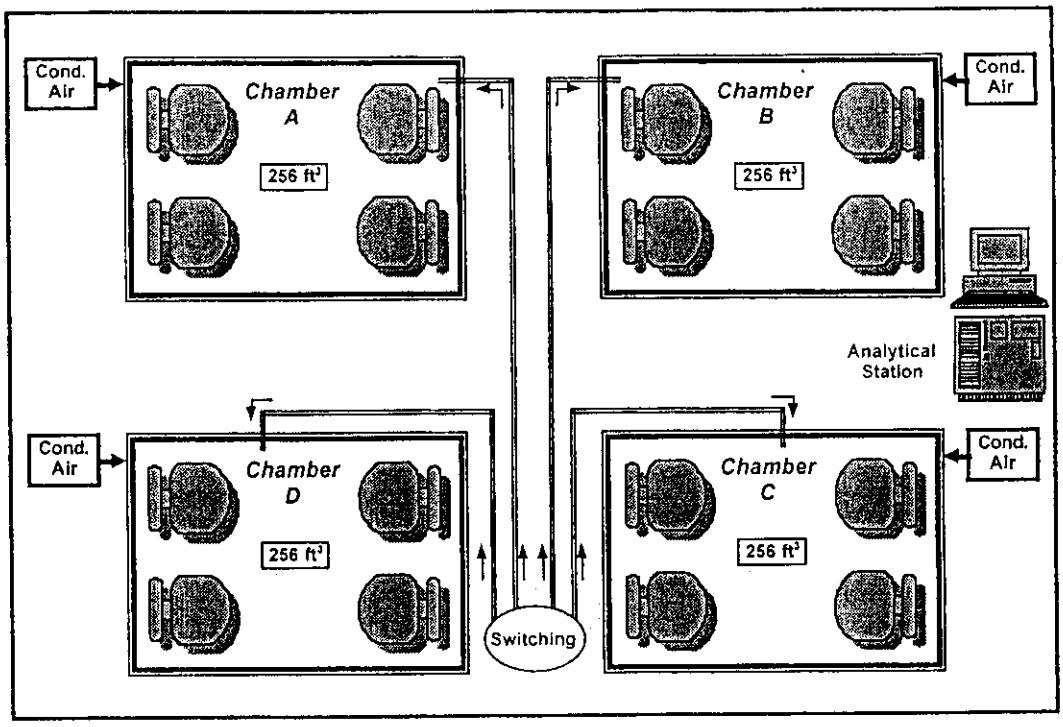


Figure 4. Showing the chambers to be used for exposures in Phases 2 and 3.

Procedure: In brief exposures, subjects will be exposed to the air in the chamber for 5 sec at a time and make a judgment regarding whether or not the chamber appears to contain test material. This will be done with a rating of confidence on a paper ballot: "Is test material present? *Yes* or *No*. Rate confidence from 0 = no confidence, to 5 = high confidence." In addition, subjects will be asked to note through which site(s) they perceived the test material.

For all testing in the chambers, the subject will wear a full respirator covering the mouth, nose and eyes until told to remove it. The reason for the device is that opening and closing the door to a chamber to let in subjects will disturb its atmosphere and subjects will need to be in the environment for a time for the atmosphere to achieve steady state. (Subjects will be fitted with appropriate respirators and taught how to keep them snug. We will test goodness of fit of the respirators in a session before any exposure. It should be borne in mind that the levels will be relatively difficult to detect even without a respirator.) Four subjects facing away from one another can occupy a chamber at once. All four chambers can have four occupants in these circumstances and after a trial has been completed the occupants can move into another chamber and repeat the process. The chamber with the test material can be varied after every fourth exposure and, on that occasion, the concentration changed. This will occur over a series of concentrations until detection is consistent. (Pilot work will lead to the appropriate range and steps.) The information about which chamber contains the test material and the level of the test material will be unknown to the person who deals with the subjects, i.e., the

study will be double blind. We have built a switching device that will feed the chambers appropriate levels of vapor and we will control flow as necessary via a feedback loop.

For the brief duration, subjects will not need to track their sensations while in the chamber, but for the long duration, they will rate irritation periodically throughout the exposure. Nevertheless, the judgment of confidence regarding whether the chamber had the test material will still be the judgment of most interest for it can be compared to whether or not test material was present.

Each subject will be tested at four to five concentrations twice. We anticipate that this can be accomplished in one session per subject for the short duration and two to three sessions for the long duration.

Data Analysis: The outcome of this testing will be the subjects' ratings of confidence in the cases when the test material is present vs. when it is absent. The data can be analyzed via ANOVA for its interval properties, i.e., actual ratings, in order to determine the concentration that can just be discriminated from blank air. The data can also be analyzed for its nominal properties, i.e., correctness vs. incorrectness, to construct receiver operating characteristic (ROC) curves to show level of discrimination. The confidence ratings will be analyzed to test the hypothesis that time alters sensitivity.

Phase 3: Signs/Symptoms of Irritation in Daily Exposures

Objective: To establish whether mildly irritating ambient exposures of one-half hour per day over four days lead to a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The level of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways.

The objective will be addressed in two stages, Phase 3a and Phase 3b.

Phase 3a - Preliminary Study to Validate Techniques of Measurement: Phases 1 and 2 will establish the relative sensitivity of the nose, eyes, and throat to chloropicrin. Phase 2 will establish whether the ability of chloropicrin to provoke irritation cumulates over periods up to a half-hour in ambient exposures. The stimulus-response functions generated in those studies will inform the choice of test concentrations in Phase 3. However, to address Aim 3 we will need additional information obtainable only via a preliminary study. It will serve to establish: a) sensitivity and stability of clinical assessment of signs and symptoms, b) sensitivity and stability of three assays for biochemical markers, and c) sensitivity and stability of the Rhinconjunctivitis Quality of Life Questionnaire in the context of the present work (e.g., Juniper, Thompson, Ferrie, & Roberts, 2000). Statistically speaking, the preliminary study deals with issues of the power to detect effects of exposure to chloropicrin when these exist, but the preliminary study of Phase 3a will entail no exposure to chloropicrin. It will instead set the stage for exposure to chloropicrin in Phase 3b.

Clinical Studies of Rhinitis: In some respects, the agenda for Phase 3 resembles that of clinical studies of effects of medication on allergic rhinitis. In view of the

uniqueness of the present work, the similarities to and differences from those clinical studies seem worthy of comment. In such studies, investigators may challenge subjects with allergens in order to induce reactions and may measure how medication diminishes sensitivity or reactivity. Outcome variables of interest generally include a score for signs/symptoms and perhaps an index of quality of life, such as the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), and some objective indices, such as biochemical markers of inflammation. Variables of this nature have relevance to the present research. In the present case, however, a major question concerns whether subjects will become symptomatic and show signs of rhinitis from environmentally plausible exposures rather than whether they will become less symptomatic from medication. If they become symptomatic, then the outcome variables need to show the result and its various manifestations with clarity. If they do not, then the investigators need to show that the outcome variables would have registered a positive result if it had occurred. This accounts for concerns over sensitivity and stability.

Blinding and a "Reverse Placebo:" The present study differs from clinical trials not only in that the agent of interest may induce symptoms, but also in: a) the inability to blind subjects to presence of the agent, a noxious vapor, b) absence of a reliable way to provoke rhinitis in normals and reluctance to do so needlessly, and c) the time-frame of the effects. In the clinical trials, investigators normally compare subjects treated with the medication, these days typically a topical agent, against those given a placebo. In some instances, such as some studies of seasonal allergic rhinitis, the investigators may not provoke symptoms in the laboratory but may rely upon everyday exposures to keep the subjects symptomatic. In those cases, the investigators gather data before treatment and compare it with data gathered during a regimen of treatment. Even though the studies have the opportunity to use subjects as their own controls, the studies will normally include two groups, one treated with active ingredient and one given placebo. A greater reduction in signs/symptoms in the group treated with active ingredient counts as success (e.g., Van Cauwenberge, Juniper, & the STAR Investigating Group, 2000). In such studies, the subjects who receive the placebo commonly show some subjective improvement, i.e., reduction in symptoms (see Kobayashi, Beaucher, Koepke, Luskin, et al., 1995; Meltzer, Jalowayski, Orgel, & Harris, 1998). If the studies measured effects before vs. after the medication only, the outcome would inflate the benefits of the medication. In the same manner, studies of exposure to an irritating vapor may inflate symptoms unless they use the equivalent of a "placebo." At this point, we can only speculate about whether this will prove true.

Whereas investigators in the clinical trial can blind subjects to the presence or absence of the active ingredient, investigators of a noxious exposure cannot. As an approximation, though, the investigators can expose subjects to a vapor that precipitates no actual irritation, but at least stimulates the same mucosal tissue as an irritating vapor. In the present case, we have chosen a material known as WS 3, a non-irritating, odorless cooling agent used in consumer products, as this type of "reverse placebo." Its use in Phase 3a will serve to examine the lability of subjects' symptoms as part of the investigation of sensitivity and stability of the ratings of symptoms.

Positive Control Group vs. Positive Control Exposure: Clinical studies provoke symptoms and signs in allergic subjects under conditions well characterized with respect to agent and duration of effect. Hence, when an investigator sprays a dilute solution of ragweed pollen extract into the nose of a person with known allergy to that substance, he can anticipate that an ensuing acute episode may begin within minutes and subside after an hour. The protocol may even entail increasing the concentration of the challenging agent until a response occurs. This establishes the positive control. The study of a noxious vapor cannot easily follow this simple route. It is unclear how to provoke rhinitis in a normal person. An irritating vapor may do so, but at this time we know neither the vapor nor the level that will do so unfailingly. Nor can we know how to provoke the symptoms as temporarily as one might in persons with allergic rhinitis. How then can one demonstrate the sensitivity to resolve the presence vs. the absence of rhinitis in the study of a noxious vapor? One way would be to demonstrate the ability of the outcome measurements to resolve between normals and persons already symptomatic. For this, one can study persons screened for normal nasal health and persons screened for presence of symptomatic allergic rhinitis. By this device, a positive control group substitutes for a positive control exposure.

Ratings: In clinical studies of rhinitis, investigators rather commonly ask subjects to fill out simple ratings of symptoms such as that shown in Table 1 below. The scale reportedly picks up differences of approximately half a step in nasal score with power greater than 90% in groups of a dozen or so subjects, but the published literature contains little documentation of such sensitivity (Meltzer, personal communication).

Although indispensable in clinical studies, ratings of symptoms fail to capture the effects of symptoms on everyday life. This situation has given rise to questionnaires that assess quality of life. The RQLQ, a self-administered questionnaire of high reliability (Cronbach's alpha >0.90) and validity, assesses quality of life as pertains to the nose and eyes. Via a series of 28 questions it assesses how "nose/eye symptoms trouble you in your life." Regarding sleep, for instance, it inquires: "How troubled have you been by each of these sleep problems during the last week as a result of your nose/eye symptoms? 4. Difficulty getting to sleep. [The respondent has six choices per item ranging from *not troubled* to *extremely troubled*] 5. Wake up during night. 6. Lack of a good night's sleep." Most commonly, ratings of quality of life ask the respondent to consider the previous six or seven days. In general, the RQLQ is not given on sequential days, but we see no a prioi reason why it would not work well even though many days of the frame of reference, a week, would overlap.

Clinical studies of rhinitis include ratings from clinicians as well as ratings from subjects. Although the ratings of the clinicians include signs, such as congestion of the nose and tearing of the eyes, they also include the impressions of the subjects. Consequently, it hardly surprises that the ratings of the subjects and the ratings of the clinicians agree extremely well (see Meltzer et al., 1998). In a study that cannot blind the subject from the nature of an exposure, it seems essential to blind the clinician. The clinician can use a rating scale not unlike that of the subject (Table 2), but should remain oblivious to conditions of exposure to remain unbiased.

Biochemical Markers: Irrespective of whether it arises from allergies, infection, or irritation, rhinitis represents an inflammation of the nasal mucosa. The presence of inflammation reveals itself via the type and number of cells that can be sampled from the mucosa. In samples taken by Rhinoprobe and quantified as number of cells per high power field (HPF), normal superficial nasal mucosa has the following cytologic profile: neutrophils 0-10.5, eosinophils 0-0.45, and basophilic cells 0-0.2 (Jalowayski & Zeiger, 1988). When neutrophils reach 16-20, eosinophils reach 1.1-5.0, and basophilic cells reach 0.4-1.0, subjects characteristically exhibit medically significant inflammation.

The presence of inflammation in mucosal tissue can also reveal itself via levels of biochemical markers. Those shown to increase significantly in nasal and conjunctival mucosa, nasal secretions, and tear fluid after challenge with allergens include albumin, interleukin-8 (IL-8) and soluble intercellular adhesion molecule (sICAM) (Ciprandi, Pronzato, Passalacqua, Ricca, et al., 1996; Calderon, Devalia, Prior, Sapsford, & Davies, 1997; Granstrand, Nylander-French, & Holmstreom, 1998; Wilson, Lau, & Howarth, 1998). Albumin leaks across the mucosal layer during an inflammatory process. Its concentration in nasal fluid and tears collected via absorbent sponge can be measured by ELISA. IL-8, one of several proinflammatory cytokines that play a major role in attracting and activating inflammatory cells, can also be measured by ELISA. sICAM, known to play a role in eosinophil and neutrophil infiltration across endothelial and epithelial cells, can also be measured by ELISA.

The accuracy of research on markers in secretions can depend upon the mode of collection, a constantly evolving matter as investigators abandon error-prone methods. The present research will entail collection of fluid with small cellulose sponges. Such collection does not irritate and avoids uncertainties regarding dilution as in the method of nasal lavage. Weight of the sponge before and after 30-sec application to the mucosa gives an exact measure of amount of fluid collected. The sponge also elutes albumin better than filter paper, another medium of collection. Because collection via the sponge is new, few data address sensitivity to analytes except for eosinophilic cationic protein (ECP) and tryptase in secretions (Klimek, Wolf, Mewes, Dormann, et al., 1999). We believe that we will collect sufficient quantities of nasal and tear fluid to measure levels of albumin, IL-8, and sICAM accurately. The preliminary study will test ability to resolve between normals and symptomatic persons.

Preview of the Preliminary Study: The study described in its particulars below will entail the following: Two groups of subjects, one screened for absence of nasal inflammation and the other for presence of allergic rhinitis will participate in eight half-hour sessions, a block of four on successive days that will entail exposure to air and another block of four on successive days that will entail exposure to a cooling level of WS 3. Half the subjects per category will have the exposure to air first and half the exposure to WS 3 first. In neither case, should the subjects experience irritation from the stimulus. Subjects will have a clinical exam of the nose, eyes, and throat before each exposure and twice after the exposure, within a half-hour and after another hour. At the approximate times when subjects will have the clinical exams, they will also rate their symptoms. In connection with the clinical exams, the examiner will collect fluid from the nose and eyes for analysis of the markers albumin, IL-8, and sICAM. Before an

exposure and 24 hours after the fourth exposure in a block, the subject will fill out the RQLQ with respect to the previous 24 hours.

Questions addressed in the preliminary study will include:

1) How well will a blinded clinical exam resolve between the normals and the subjects with rhinitis? We expect that the exam will resolve between the groups, but the variability day to day, across groups, and across exposures should yield important statistical information about the sensitivity and stability of the exam.

2) How much lability will subjects show in their ratings of symptoms? Will the ratings differ between exposures to air and exposures to cooling agent? The answer may have implications for how to control against biased ratings in studies that cannot blind subjects to exposures. The study will also, however, offer statistical information about the sensitivity and stability of the particular ratings of symptoms (Table 1). How, one might ask, can we know that the cooling agent does not cause irritation and that any increase in symptoms does not occur because of actual irritation? The clinical exam can serve as arbiter here. Moreover, quite possibly the subjects with allergic rhinitis might have their symptoms reduced by exposure to the cooling agent.

3) Will the RQLQ perform meaningfully for resolution between normals and persons with rhinitis when given repeatedly?

4) Will secretions collected with sponges provide enough material for reliable and sensitive assays for the markers albumin, IL-8, and sICAM?

Stated as an objective, Phase 3a entails the following:

Objective: To establish in 32 subjects (16 males and 16 females), half of them (eight males and eight females) screened for normal mucosal condition and the other half for allergic rhinitis, the stability and sensitivity of various to reflect the presence of mucosal inflammation.

Table 1

Rating of Symptoms

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Collected by:_____

Use the following scale to indicate the degree of symptoms:

Scale	Degree	Meaning
0	None	No symptom.
1	Mild	Symptom present, but minimal awareness, easily tolerated.
2	Moderate	Symptom definite and bothersome, but tolerated.
3	Severe	Symptom hard to tolerate and can interfere with activities of daily living or sleeping.

Nose

a. Congestion ____
b. Runny Nose ____
c. Itchiness/Sneezing ____
d. Irritation ____ ____

Eye

a. Tearing ____
b. Puffiness ____
c. Itchiness ____
d. Irritation ____ ____

Throat

a. Cough ____
b. Hoarseness ____
c. Dryness ____
d. Irritation ____ ____

Total Score ____

Table 2

Rating of Signs

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Examined by:_____

Use the following scale to indicate the degree of clinical signs:

Scale	Degree
0	No sign evident.
1	Sign barely present.
2	Sign clearly present.
3	Sign quite marked.

Nose

a. Congestion	____	
b. Rhinorrhea	____	
c. Erythema	____	____

Eye

a. Tearing	____	
b. Puffiness	____	
c. Erythema	____	____

Throat

a. Cough	____	
b. Swelling	____	
c. Erythema	____	____

Total Score ____

Apparatus: Exposures will take place in the chambers, with concentration of WS 3 (n-ethyl-5-methyl-2-(1-methylethyl)-cyclohexanecarboxamide, CAS 39711-79-0) controlled in the manner shown for Phase 2, i.e., vaporization of liquid injected onto a warmed surface. Other apparatus will include that used in screening (see details under **7. Human Subjects**).

Procedure: Screening will establish two groups of subjects, 16 with and 16 without evidence of nasal inflammation (see details under **7. Human Subjects**). Every subject will participate in the same tests that will revolve around eight exposures of one half-hour in the chambers. Four exposures will take place in one block of four days and four in another block, with a minimum four-day break between blocks. In one span of four days, subjects will have exposure to WS 3 at a just-cooling level and, in the other span, those same subjects will have exposure to just air on the four successive days. Half the subjects will have exposure to WS 3 first and half exposure to air first.

On each day of exposure, the following will be performed before exposure begins (details of exams appears under **7. Human Subjects**):

1) subject fills out the RQLQ,

2) subject rates symptoms (see Table 1),

3) clinical examination of the nose, eyes, and throat, and rating of signs (seeTable 2),

4) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, and

5) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min.

The following will then be performed in the chamber:

1) subject enters with a full-face respirator in place while vapor concentration is established and removes the respirator on cue to begin half-hour exposure, and

2) in the last half-minute of exposure, the subject rates symptoms.

Fifteen min after exposure, the following will be performed:

1) subject rates symptoms,

2) clinical examination of the nose, eyes, and throat, and rating of signs,

3) sample of nasal secretion taken, as before exposure, and

4) sample of tear fluid taken, as before exposure.

This procedure will be repeated during the four days of exposure in a series. On the day after the fourth exposure of a series, the subject will fill out an RQLQ and return it by mail or phone in the answers.

Data Analysis: Ratings of symptoms (scores per site and total score) will be analyzed for reliability, for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis, and for influence of exposure to WS 3 vs. air.

Score on the ROLQ will be analyzed for reliability, for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis, and for influence of exposure to WS 3 vs. air.

Ratings from the clinical exam will be analyzed similarly as the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions will be assessed for the markers albumin, IL-8, and sICAM. The quantities will be compared for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

As noted at the outset, this study concerns statistical power. From a practical standpoint with respect to Phase 3b, these analyses will boil down to assessment of the number of subjects needed to establish meaningful effects in the study of exposure to chloropicrin.

Phase 3b - Daily Exposures, Signs and Symptoms of Irritation, and Evidence of Inflammation: The work in Phase 3b will build upon that of Phase 3a, but will entail exposure of subjects to chloropicrin.

Objective: To establish in 16 males and 16 females, screened for normal mucosal condition, whether mildly irritating ambient exposures to chloropicrin of one-half hour per day over four days lead to: a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The levels of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways. (The numbers 16 males and 16 females may require modification since results of Phase 3a will determine the number of subjects needed for criterion levels of power.)

Apparatus: Exposures will take place in the chambers. Concentration will be controlled as indicated above in Phase 2. Other apparatus will include that used in screening.

Exposures will entail two concentrations of chloropicrin and a blank. The lower concentration of chloropicrin will equal 0.1 ppm, the occupational threshold limit value (TLV). The higher concentration will equal that detectable on approximately 75% of trials in Phase 1. Most likely, this will occur at about 0.15-0.20 ppm.

Procedure: Subjects will serve in three blocks of six sessions, each beginning on a Friday and ending on a Friday. Exposures to a given concentration in the chambers will last one half-hour on four successive days, Monday through Thursday. In one block, a subject will have exposure to chloropicrin at 0.1 ppm. In another block, the subject will have exposure to a just-irritating level, as indicated above, and, in a third block, the subject will have exposure to just air on the four days. The order in which a given subject has exposure to the three conditions will vary to prevent confounding of order of exposure with level. Except for personnel who set and monitor the conditions in the chamber, the personnel who will deal with the subjects and the subjects themselves will be blinded to the conditions at any given time. At least one week will separate the end of one block and the beginning of another for a subject.

On the first Friday of a block, baseline measures will be taken (see Table 3). The following will be performed:

1) a Rhinoprobe sample taken from the inferior turbinate in one nostril to establish the number and kind of cells in the mucosal layer (see details under **7. Human Subjects**),

2) subject fills out the RQLQ,

3) subject rates symptoms (see Table 1),

4) clinical examination of the nose, eyes, and throat, and rating of signs,

5) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, and

6) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min,

On Monday through Thursday, the following will be performed before the subject enters the chamber:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination of the nose, eyes, and throat, and rating of signs,

4) sample of nasal secretion taken.

5) sample of tear fluid will be taken,

6) office spirometry to establish forced vital capacity (FVC) and forced expiratory volume at 1 sec (FEV_1),

7) exhaled nitric oxide (NO) measured via the mouth while exhaling through an expiratory resistance to indicate NO generated in the lungs, and

8) nasal resistance measured by active, anterior rhinomanometry.

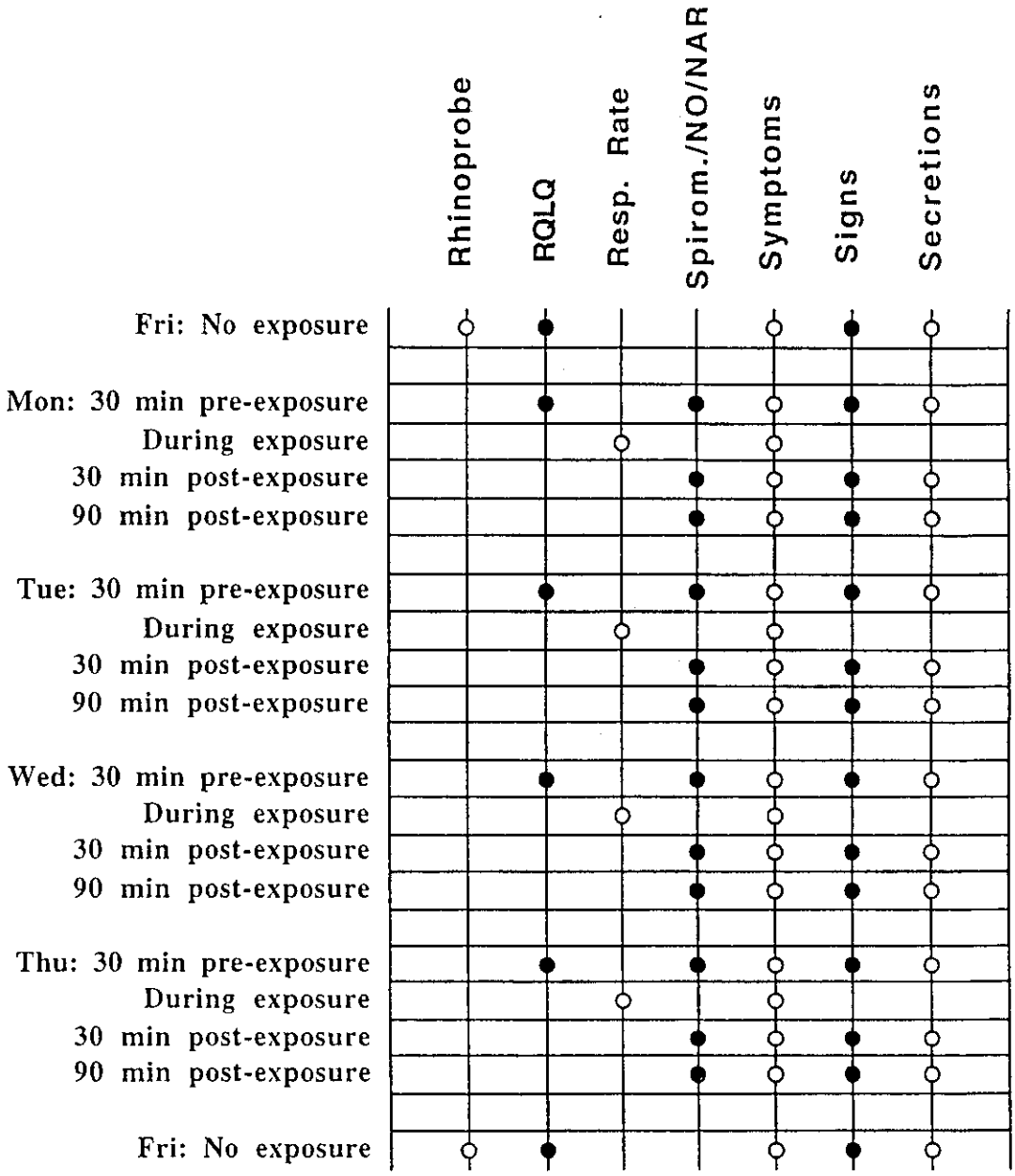
The following will then be performed in the chamber:

1) subject enters with a full-face respirator in place while vapor concentration is established and removes the respirator on cue to begin the half-hour exposure,

2) respiratory rate monitored remotely via Respibands placed around the thorax, and

3) in the last half-minute of exposure, the subject rates symptoms.

Table 3


Rhinoprobe
RQLQ
Resp. Rate
Spirom./NO/NAR
Symptoms
Signs
Secretions
Fri: No exposure
Mon: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Tue: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Wed: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Thu: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Fri: No exposure

Thirty min and 90 min after a session, the following will be performed:

1) subject rates symptoms,

2) clinical examination, with rating of signs,

3) nasal secretion taken, as before the session ,

4) tear fluid taken, as before the session,

5) office spirometry, as before the session,

6) measurement of nasal resistance, as before the session, and

7) exhaled NO measured, as before the session.

On Tuesday, Wednesday, and Thursday, the cycle will be repeated, i.e., a pre-session evaluation and two post-session evaluations.

Approximately 24 hr after the Thursday session, the subject will return and the following performed:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination, with ratings of signs,

4) nasal secretion collected, and

5) Rhinoprobe sample taken from the opposite nostril from the first.

Data Analysis: For interpretation of the study, we will distinguish between variables meant to monitor safety and those of substantive interest.

With respect to safety:

Respiratory rate will be monitored to examine whether the subject remains relaxed during exposures. An unexpected rise can indicate anxiety and gives reason to query the subject about any perceived threat.

Results of the office spirometry will be compared before and after exposure to examine whether a subject has experienced bronchoconstriction from the exposure. A reduction of FEV_1 of 15 % will be reason to remove a subject from further exposures at the level that caused the constriction.

Exhaled nitric oxide (NO) will be compared between after and before to examine whether a subject has developed any inflammation in the lungs.

With respect to substantive interest:

Score on the RQLQ will be analyzed for effects of level of exposure (0 ppm, 0.1 ppm, >0.1 ppm), of day as an indicator of total exposure, and of sex.

Ratings of symptoms (scores per site and total score) will be analyzed for effects of level of exposure, of time since exposure (30 min and 90 min post-exposure), of day as an indicator of total exposure, and of sex.

Ratings of signs on the clinical examination will be analyzed similarly to the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions from both the nose and the eye will be assessed for total mass collected and for the markers albumin, IL-8, and sICAM. The quantities of the biochemical indices will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

Nasal resistance will be compared before vs. after exposure as an aspect of dose of chloropicrin vapor. A blocked nasal passage means lower dose than a patent passage. An increase in resistance is a normal response to inhalation of an irritating vapor. Nasal resistance will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex.

The cells in the Rhinoprobe samples will be compared from the first to the sixth days as an index of cumulative effect of exposure.

7. Human Subjects

Inclusion Criteria for Normal Subjects:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

4) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Normal Subjects:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses. (Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)

8) Abuse of alcohol (more than three drinks per day) or drugs.

9) Smoking of tobacco or marijuana within the previous year.

10) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

11) Impaired sense of smell.

12) Pregnancy at the time of the study.

13) Evidence of active infection, rhinitis, pharyngitis, clinically-significant inflammation in the nose or throat, or certain structural abnormalities in these regions.

14) Evidence of conjunctivitis, abnormal redness of the eyes, or abnormalities of the surface of the eyes.

15) Clinically elevated nasal resistance.

16) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of any of these conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

Sense of Smell: Screening for smell will entail taking a standardized test, e.g., the Connecticut Chemosensory Clinical Research Center [CCCRC] Test for odor threshold and odor identification (Cain, 1989). Subjects will excluded if their olfactory ability falls below normal by the criteria of the test.

Nasal Resistance: The assessment of nasal resistance will occur both in screening and in testing in Phase 3b. Nasal resistance will be measured via a system of computerized anterior rhinomanometry (RHINO; MultiSpiro, San Clemente, CA) that avoids deformation of the nares. The system relies upon an oxygen-type face-mask to monitor flow from one nostril while a tube sealed via a pressure patch (Rhino Diagnostics, Inc., San Diego, CA) monitors pressure at the other nostril.

During testing, the subject breathes normally through the mask through four cycles per nostril. Signals for pressure and flow are digitized and used to calculate resistance at –1.5 cm water column. Subjects with clinically abnormal resistance, defined as >5 cm H_20/ L/sec will be excluded in screening.

Nasal Cytology: Nasal cytology will be used both in screening and in testing in Phase 3b. Subjects who pass the clinical screening examination of the nose, throat, and eyes may still show evidence of inflammation in cytological analysis. In order to obtain cells, the investigator will use a Rhinoprobe, a flexible curette with a 1-mm diam. cup. Sampling with the Rhinoprobe entails a gentle scrape of 3-mm length along the superficial nasal mucosa of the lower turbinate under visual inspection. The procedure causes minor discomfort for an instant.

For analysis, the specimen is gently spread over a small area of a microscope slide, fixed in 95% alcohol, and stained with Wright-Giemsa stain. It is examined under low power (100 x) to determine the adequacy of the specimen and the areas of interest and then graded under high power (1000 x) for cells.

Nasal cytology can reveal various conditions, as follows (see Table 4): 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection, 3) large numbers of eosinophils or basophilic cells (3+ or 4+) provide evidence of inflammation. When used as an outcome variable in testing in Phase 3b, cells will be counted exactly.

Spirometry: Spirometry will be used both in screening and in testing in Phase 3b. Office spirometry testing will conform to the recommendations of the American Thoracic Society. Data to be acquired from this test include the following: FVC, the total volume of gas exhaled after a full inspiration, and FEV_1, the gas volume exhaled in one second by a forced expiration from a full inspiration. The data will allow calculation of the ratio FEV_1/FVC. Subjects whose pulmonary function fails to lie at or above 75% of predicted FEV_1 or FVC will be excluded.

Ocular Cytology: Ocular cytology will be performed in screening on impression-samples taken from the conjunctival membrane inside the lower eyelid. The lower lid

will be sampled with a 3-mm diam. membrane filter placed at the end of a rod. The rod weighs 60 g and suspension of the rod within a short outer cylinder at the moment of contact insures that 60 g will be exerted on the lid. The samples will be analyzed for the presence of cells in the same way as in nasal cytology.

Table 4
Guide for Grading Nasal Cytograms

Quantitative Analysis	Semi-quantitative Analysis	Grade
Epithelial Cells		
N/A	Normal morphology	N
N/A	Abnormal morphology	A
N/A	Ciliocytophthoria	CCP
Eosinophils, neutrophils		
0*	None	0
0.1-1.0*	Occasional cells	0.5+
1.1-5.0*	Few scattered cells or small clumps	1+
5.1-15.0*	Moderate number of cells and larger clumps	2+
15.1-20.0*	Larger clumps of cells which do not cover the entire field	3+
>20*	Large clumps of cells covering the entire field	4+
Basophilic cells		
0*	None	0
0.1-0.3*	Occasional cells	0.5+
0.4-1.0*	Few scattered cells	1+
1.1-3.0*	Moderate number of cells	2+
3.1-6.0*	Many cells easily seen	3+
>6.0*	Large number of cells, as many as 25 per high power field	4+
Bacteria**		
N/A	None seen	0
N/A	Occasional clump	1+
N/A	Moderate number	2+
N/A	Many easily seen	3+
N/A	Large numbers covering the entire field	4+
Goblet cells***		
0	None	0
1-24%	Occasional to few cells	1+
25-49%	Moderate number	2+
50-74%	Many easily seen	3+
75-100%	Large number, may cover entire field	4+

* Mean of cells per 10 high power fields (x1000)
** Note presence of intracellular bacteria
*** Ratio of goblet cells to epithelial cells, expressed as percentage

Persons with significantly elevated levels of PMNs (neutrophils, eosinophils, and basophilic cells) in the conjunctival membrane will be excluded. Persons with small elevations above normal will not.

Pregnancy: Testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded.

Inclusion Criteria for Subjects with Allergic Rhinitis:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must show evidence of allergic rhinitis.

4) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

5) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Subjects with Allergic Rhinitis:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses. (Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)

8) Abuse of alcohol (more than three drinks per day) or drugs.

9) Smoking of tobacco or marijuana within the previous year.

10) Daily use of medication, excluding birth control pills.

11) Absent sense of smell.

12) Evidence of active infection or certain structural abnormalities in the upper airways.

13) Pregnancy at the time of the study.

14) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of signs of allergic rhinitis should be present in the patients. Signs of other conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

As part of the examination, the examiner will also establish if there is a history of seasonal or perennial allergic rhinitis with positive skin test to one or more allergens within previous 12 mo. If skin test results are unavailable, a test will be performed at the Nasal Dysfunction Clinic under the supervision of Dr. Terence Davidson. Positive evidence will be a criterion for inclusion. The examiner will also ask about symptoms. A score ≥ 8 for the combined nasal and eye symptoms, with congestion score ≥ 2 for at least three of the five days prior to screening will be a criterion for inclusion.

Sense of Smell: Screening for smell will entail the same testing as described for normals. Subjects will excluded if their olfactory ability falls into the anosmic zone, i.e., if the subject evinces no olfactory ability whatsoever.

Nasal Resistance: The assessment of nasal resistance will entail the same testing as described above. Subjects will be excluded if their resistance lies above 6 H_2O/L/sec.

Nasal Cytology: Nasal cytology will be performed as described above. Subjects will be excluded if they show the following in their cytograms: 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, or 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection.

Spirometry: Screening will entail the same methods as described for normals.

Ocular Cytology: Screening will be performed by the methods described above for normals. Subjects will not be excluded by this test, except for signs of infection, but the results will be compared with those of the nasal cytology for possible stratification of the sample of subjects by presence or absence of inflammatory cells in the eye.

Pregnancy: Testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded.

8. Informed Consent

Subjects will be recruited from the general population. Formal consent will be sought just prior to screening, typically by staff involved in the screening tests. The consent form will contain the relevant facts regarding the reason for the study, the task at hand, any possible adverse effects, and the opportunity to withdraw at any time without penalty. Consent will be written and the subject will receive a copy of the form along with the subject's bill of rights.

9. Therapeutic Alternatives

Not a therapeutic study.

10. Potential Risks

Screening: The screening procedures of direct visualization of the nasal and ocular mucosae and measurement of nasal resistance pose no realistic risks that we can anticipate. Sampling from the nasal mucosa has been performed routinely by Dr. Jalowayski in the Nasal Dysfunction Clinic for many years and entails minimal risk. The impression cytological sampling from the lower lid, also entails minimal risk.

Testing: The risks associated with testing entail sensory irritation of the upper airways or eyes and remotely in these circumstances asthma. We say "remotely" because the exposures will be to perithreshold concentrations with respect to irritation.

11. Risk Management

Monitoring: Subjects will be monitored visually during testing. Testing will stop if the subject finds the test situation uncomfortable and asks to stop. We will instruct subjects to report any respiratory symptoms immediately.

Actions to be taken if an adverse event occurs:

Upper Airway or Ocular Discomfort: Irritation of the nose or eyes should subside soon after exposure. Should effects last longer than expected, the subject will be instructed regarding how to irrigate the affected area with saline solution. An eye irrigation system exists within the laboratory. Furthermore, over-the-counter preparations for such purposes will be available on-site for the subject to use before leaving the lab and to take home. The subject will be informed that if any such irritation or discomfort fails to show progressive improvement during the ensuing hours, then the subject should page Dr. Terence Davidson who will oversee medical management of the symptoms.

Lower airway reactions: Should exposure cause shortness of breath from apparent bronchoconstriction, then an ACLS-certified staff member will initiate an asthma-management protocol. (This person, to be hired, will have training appropriate to obtain ACLS certification. Candidates will be nurses, respiratory therapists, and EMTs.) An emergency kit to treat acute asthma will include: a) a Ventolin inhaler (albuterol USP

inhalation aerosol). b) an AeroChamber to be used with the metered dose inhaler, c) a nebulizer and oxygen source. d) a pulse oxymeter. and e) an epinephrine auto-injector (EpiPen) to deliver a 0.3 mg intramuscular dose of epinephrine (1:1000), and d) apparatus for oxygen therapy.

The protocol for management of acute asthma will be the following: a) if symptoms occur, the subject will be offered treatment with Ventolin (one puff held in the lungs for 10 sec., followed by a second puff 30 sec later), b) the pulse oxymeter will be attached to a digit in order to monitor oxygen saturation, c) if the subject seems unable to inhale from the inhaler adequately, then the nebulizer will be used, d) Dr. Thomas Bruff will be paged and informed of the event, and he will decide how the subject should be managed, e) if the subject obtains relief within 15 min. and Dr. Bruff gives no instructions to the contrary, the subject will be asked to remain in the lab for the next half-hour or until he no longer feels short of breath; he will be called at intervals over the next 8-24 hr to inquire about any late reaction, f) if the subject fails to obtain relief, he will be transported to the emergency room at Thornton Hospital, less than 2 miles from the lab. If the subject shows signs of anaphylactic shock, he can be given an injection of epinephrine from the EpiPen and administered humidified oxygen.

Regarding privacy, personal identifiers such as names or Social Security numbers will not be included in any report. Only the investigators, study sponsor and state and federal regulators will have access to the raw data, unless required by a subpoena.

12. Potential Benefits

There will be no benefits to individual subjects.

This research is motivated by the need to develop a definitive set of data relevant to environmental and occupational exposures to chloropicrin. Since the chemical has very low systemic toxicity compared to its rather strong irritating properties, it is irritation that will dictate permissible exposure levels. (The irritation response, as we have noted, largely protects against systemic exposure.) In so far as the present research makes it possible to set more scientifically defensible exposures, then its potential benefit to occupational and public health is considerable.

Approximately 50% of the ACGIH TLV's are based on sensory irritation and yet there have been almost no controlled studies of such. We seem to be entering a new era when companies and trade groups that exercise product stewardship over commodity chemicals seek quantitative data on human sensory irritation. As product stewards, these entities are often asked to advise users of their products about hazardous properties. Indeed, they must do so in Material Safety Data Sheets. This matter goes beyond merely setting permissible exposure levels into more intrinsic understanding of the perceptual and physiological effects of the chemicals. We see these developments as salutary.

13. Risk-Benefit Ratio: Minor risk. no individual benefits. but benefits to society as indicated just above.

14. Expense to subject: None anticipated.

15. Payment for Participation: Subjects will receive $15 per hour for participation. subjects can receive their payment (cash) at the end of a session or can allow it to accumulate over sessions by mutual agreement. Subjects will not be reimbursed for travel.

16. Privileges/Certifications and Licenses: Dr. Davidson has privileges at UCSD Medical Center. He indicated to the Human Subjects Program in 1999 that sampling from mucosal tissue, the only procedure where we touch the subject, does not require direct medical supervision. Dr. Jalowayski, who has worked with Dr. Davidson in the Nasal Dysfunction Clinic for more than 15 years, developed the sampling and performs it routinely in the clinic. Dr. Jalowayski will do the sampling in this project.

Dr. Thomas Bruff, board certified in internal medicine and in occupational medicine, is attending staff at UCSD Medical Center. He is ACLS-certified.

17. Bibliography:

American Conference of Governmental Industrial Hygienists (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th ed. Cincinnati: ACGIH.

Cain, W. S. (1989). Testing olfaction in a clinical setting. Ear, Nose, and Throat Journal, 68, 316-328.

Calderon, M. A, Devalia, J. L, Prior, A. J, Sapsford, R. J, & Davies, R. (1997). A comparison of cytokine release from epithelial cells cultured from biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis..Journal of Allergy and Clinical Immunology, 99, 65-76.

Ciprandi, G., Buscaglia, S., Pesce, G., Villaggio, B., Bagnasco, M., & Canonica, G.W. (1993). Allergic subjects express intercellular adhesion molecule – 1(ICAM-1 or CD54 on epithelial cells of conjunctiva after allergen challenge. Journal of Allergy and Clinical Immunology, 91(2), 783-792.

Ciprandi, G, Pronzato, C, Passalacqua, G, Ricca, V, Bagnasco, M, Grogen, J, Mela, G. S. & Canonica, G. W. (1996). Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells: An antiallergic activity. Journal of Allergy Clinical Immunology, 98(6), 1088-96.

Granstrand, P., Nylander-French, L., & Holmstreom, M. (1998). Biomarkers of nasal inflammation in wood-surface coating industry worker. American Journal of Industrial Medicine, 33(4), 392-9.

Jalowayski, A. A. & Zeiger, R.S. (1988). Examination of nasal or conjunctival epithelium specimens. In Manual of Allergy and Immunology, eds. Lawlor, G. J. and Fischer, T.J.; Little, Brown and Co. Boston/Toronto. Pp. 432-434.

Juniper, E. F., Thompson, A. K., Ferrie, P. J., & Roberts, J. N. (2000). Development and validation of the Mini Rhinoconjunctivitis Quality of Life Questionnaire. Clinical and Experimental Allergy, 30, 132-140.

Klimek, L, Wolf, H, Mewes, T, Dormann, D, Reske-Kunz, A, Schnitker, J & Mann, W. (1999). The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophilic cationic protein and tryptase in nasal secretions. Journal of Allergy and Clinical Immunology, 103, 47-53.

Kobayashi, R. H., Beaucher, W. N., Koepke, J. W., Luskin, A. et al. (1995). Triamcinolone acetonide aqueous nasal spray for the treatment of patients with perennial allergic rhinitis: A multicenter, randomized, double-blind, pacebo-controlled study. Clinical Therapeutics, 17, 503-513.

Krieger, R. I. (1996). An assessment of implied worker exposure and risk associated with chloropicrin loading, application, and field tarping activities following application, and implied exposure and risk for off-field concentrations resulting from soil fumigation. Registration document submitted to CAL-EPA Department of Pesticide Regulation, October 1996.

Meltzer, E.O., Jalowayski, A.A., Orgel, H.A., & Harris, A. G. (1998). Subjective and objective assessments in patients with seasonal allergic rhinitis: Effects of therapy with mometasone furoate nasal spray. Journal of Allergy and Clinical Immunology, 102 (1), 39-49.

Polito, A. J. & Proud, D. (1998). Epithelial cells as regulators of airway inflammation. Journal of Allergy and Clinical Immunology, 102(5), 714-718.

Silkoff, P. E., Robbins, R. A., Gaston, B., Lundberg, J. O. N., & Townley, R. G. (2000). Endogenous nitric oxide in allergic airway disease. Journal of Allergy and Clinical Immunology, 105 (3), 438-448.

U. S. Environmental Protection Agency (1984). Notice to Manufacturers, Formulators, Producers and Registrants of Fumigant Products. PR Notice-84-5.

Van Cauwenberge, P., Juniper, E. F., & the STAR Investigating Group (2000) Comparison of the efficacy, safety and quality of life provided by fexofenadine hydrochloride 120 mg, laratadine 10 mg and placebo administered once daily for the treatment of seasonal allergic rhinitis. Clinical and Experimental Allergy, 30, 891-899.

Wagenmann, M., Baroody, F. M., Desrosiers, M., Hubbard, W. S., Ford, S., Lichtenstein, L. M., & Naclerio, R. M. (1996). Unilateral nasal allergen challenge leads to bilateral release of prostaglandin D_2. Clinical and Experimental Allergy, 26, 371-378.

Wilson, S. J, Lau, L., & Howarth, P. H. (1998). Inflammatory mediators in naturally occurring rhinitis. Clinical and Experimental Allergy, 28(2), 220-7.

18. Industry Studies: Supported by the Chloropicrin Manufacturer's Task Force. The Task Force approached the Chemosensory Perception Laboratory about the need for the information and we designed the study. The UCSD Office of Contracts and Grants Administration (Pamela Tiffany) has negotiated a contract with the Task Force. Activation of the contract awaits approval of the human subjects committee. The University will retain all patent rights to the results and is free to publish the work. The PI has no personal agreements (e.g., consultation, confidentiality) with the Chloropicrin Manufacturer's Task Force.

19. Other Funding: None.

20. Cancer Studies: Not applicable.

21. Biological Materials Transfer Agreement: No materials to be transported.

22. Investigational Drug Fact Sheet: No investigational drugs to be used.

23. Conflict of Interest: Form submitted here.

24. Nursing Staff: No impact.

25. Information Service: Will be completed.

Text for **Flyer for Human Sensory Irritation for Chloropicrin**

A. Phases 1, 2, and 3b

Topic: Subjects wanted for participation in research on perception of feel from vapors.

Who: Men and nonpregnant women in normal health, nonsmokers, 18-35years of age, who have had no colds or other infections within the previous month.

What: Subjects will need to give a medical history and go through screening (approximately 1.5 hours, with $30 payment) to establish that their noses, eyes, and airways are healthy. Those who pass screening will be offered the opportunity to participate in a number of sessions in which they will be asked to judge the presence or the intensity of the feel of a chemical vapor. There will be some low-level irritation associated with some exposures. Depending upon the phase of the study, subjects may be asked to some additional testing as occurs in the screening phase. Women will be tested for pregnancy by a urine test.

Where: Chemosensory Perception Laboratory of the UCSD Department of Surgery at the La Jolla Village Professional Center, Suite 1226, 8950 Villa La Jolla Dr.

When: Screening scheduled by mutual convenience.

Payment: $15 per hour. Subjects who pass screening can make $200 to $900 for participation, depending upon the phase.

Contact Kevin at 858-622-5830. Principal Investigator: William S. Cain, Ph.D.

B. Phase 3a

Topic: Subjects with allergic rhinitis, who currently have nasal congestion and runny noses from pollen or other seasonal allergens, wanted for participation in research on perception of feel from vapors.

Who: Men and nonpregnant women in normal health (aside from allergic rhinitis), nonsmokers, 18-35years of age, who have had no colds or other infections within the previous month.

What: Subjects will need to give a medical history and go through screening (approximately 1.5 hours, with $30 payment) to establish that their noses, eyes, and airways are healthy, except for the allergic rhinitis. Those who pass screening will be offered the opportunity to participate in a number of sessions in which they will be asked to judge the feel of a chemical vapor. Some of the testing done in screening will be repeated in connection with the exposures.

Where: Chemosensory Perception Laboratory of the UCSD Department of Surgery at the La Jolla Village Professional Center, Suite 1226, 8950 Villa La Jolla Dr.

When: Screening scheduled by mutual convenience.

Payment: $15 per hour. Subjects who pass screening can make approximately $250.

Contact Kevin at 858-622-5830. Principal Investigator: William S. Cain, Ph.D.

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010201
PENDING

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN SUBJECTS COMMITTEE

Date: March 6, 2001

To: Dr. William S. Cain Mailcode: 0957

Re: Project #010201
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

The Committee voted to approve this study pending receipt of:

1. Phases 1 & 2, 3a and 3b consents – Remove the listing of study Exclusion Criteria. This listing should occur only in the UCSD protocol, Item # 7, Human Subjects section;
2. Remove from the consents references to consent revision dates related to obtaining initial approval for a project; subsequent amendments and consent changes may include a footnote reflecting a revision date;
3. Correct the page numbering of the consents for IRB approval; and
4. Additional changes to the consent as suggested.

Please send your reply along with two copies of the revised consent (one clean copy and one copy **underlining or bolding** the changes) to the Human Subjects Program Office, 0052. Final approval will be forwarded just as soon as we can determine that your responses are satisfactory.

Sincerely,

Lucille Pearson

Lucille Pearson, Director
Human Subjects Program
0052 X44520

~~02/01/06~~

010201

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor

Phases 1 & 2

William S. Cain, Ph.D., and associates are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 25 male and 25 female subjects in the study. You will be asked to participate in approximately 5 sessions of up to 5 hours over a period of a few weeks and scheduled at mutual convenience.

In order to participate in this work, you should already have answered no regarding the following questions:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses. (Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)

8) Abuse of alcohol (more than three drinks per day) or drugs.

9) Smoking of tobacco or marijuana within the last year.

10) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

/

11) Impaired sense of smell.

12) Pregnancy at the time of the study.

PROCEDURES: If you agree to participate, the following will happen: Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), customary to the medical clinic, will be administered by Dr. Alfredo Jalowayski. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

In sessions performed after screening, your task will be to make judgments about whether you experience irritation or odor in brief exposures of your eyes or nose to just air or to very low concentrations of chloropicrin vapor.

You will receive $15 per hour for your time in screening and in testing.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort we can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of any injury caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

You may keep a copy of this consent document and "The Experimental Subject's Bill of Rights."

a copy of

You agree to participate.

_____ _____ _____

Subject's signature Witness Date

~~02/02/06~~

See letter
some changes
as Phase 1 & 2.

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to WS3

Phase 3a

William S. Cain, Ph.D., and associates are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of feel and odor from a chemical called WS3. The chemical is used commonly to impart some sense of feel in personal products. The results are intended to provide information regarding certain testing procedures we will use in later studies on chemical feel.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately 8 sessions of up to 2 hours over a period of a 2-3 weeks.

In order to participate in this work, you should already have answered no regarding the following questions:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses. (Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)

8) Abuse of alcohol (more than three drinks per day) or drugs.

9) Smoking of tobacco or marijuana within the last year.

10) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

11) Impaired sense of smell.

12) Pregnancy at the time of the study.

PROCEDURES: If you agree to participate, the following will happen: Before you will be exposed to WS3, you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), customary to the medical clinic, will be administered by Dr. Alfredo Jalowayski. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

In sessions performed after screening, your task will be to make judgments about the presence of feel from WS3, to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests.

You will receive $15 per hour for your time in screening and in testing. If you compete all testing, we estimate that you will earn an amount in the vicinity of $300.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort we can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital

costs required for diagnosis and treatment of any injury caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject. or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

You may keep a copy of this consent document and "The Experimental Subject's Bill of Rights."

a copy of

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

010201
~~02/02/06~~

Some changes as Phase 1&2

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor

Phase 3b

William S. Cain, Ph.D., and associates are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age and because you have participated in an earlier phase of this investigation. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

In order to participate in this work, you should already have answered no regarding the following questions:

Delete Exclusion Criteria from the consent.

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses. (Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)

8) Abuse of alcohol (more than three drinks per day) or drugs.

9) Smoking of tobacco or marijuana within the last year.

10) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

11) Impaired sense of smell.

12) Pregnancy at the time of the study.

If you agree to participate, the following will happen: Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), customary to the medical clinic, will be administered by Dr. Alfredo Jalowayski. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

In sessions performed after screening, we will perform various tests of how your nose and eyes respond to 30-minute exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in two six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all six days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your nose and mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15/hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $900.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort we can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late. fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute. or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of any injury caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

You may keep a copy of this consent document and "The Experimental Subject's Bill of Rights." a copy of

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

010201

COMMITTEE ON INVESTIGATIONS INVOLVING HUMAN SUBECTS
UNIVERSITY OF CALIFORNIA - SAN DIEGO

TO: Dr. William S. Cain Mailcode: 0957

RE: Project #010201
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

The above-referenced project was reviewed and approved by one of this institution's Institutional Review Boards in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56), including its relevant Subparts.

Date of IRB review and approval: **March 1, 2001**

Lucille Pearson

Lucille Pearson, Director
Human Subjects Program
0052 X44520

Note: All Human Subject research conducted at the VA facility and/or utilizing VA/VMRF funds MUST BE APPROVED by the VA Research and Development Committee prior to commencing any research.

010201

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor (Phases 1 & 2)

William S. Cain, Ph.D. and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 30 male and 30 female subjects in the study. You will be asked to participate in approximately 5 sessions of up to 5 hours over a period of a few weeks and scheduled at mutual convenience.

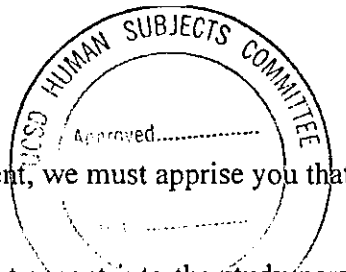
Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to make judgments about whether you experience irritation or odor in brief exposures of your eyes or nose to just air or to very low concentrations of chloropicrin vapor.

You will receive $15 per hour for your time in screening and in testing.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. Because you will be



participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

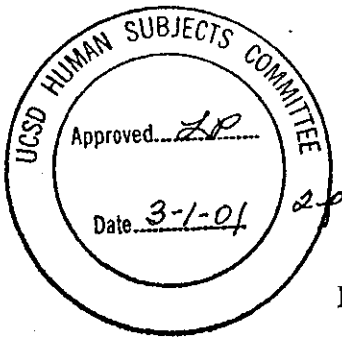
No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

010201



University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to WS3 (Phase 3a)

William S. Cain, Ph.D. and associates from the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of feel and odor from a chemical called WS3. The chemical is used commonly to impart some sense of feel in personal products. The results are intended to provide information regarding certain testing procedures we will use in later studies on chemical feel.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately 8 sessions of up to 2 hours over a period of a 2-3 weeks.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to WS3, you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by-Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

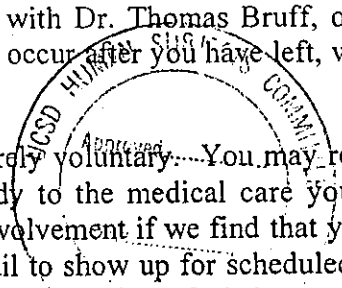
After the screening session, your task in subsequent sessions will be to make judgments about the presence of feel from WS3, to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests.

You will receive $15 per hour for your time in screening and in testing. If you compete all testing, we estimate that you will earn an amount in the vicinity of $300.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).



Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

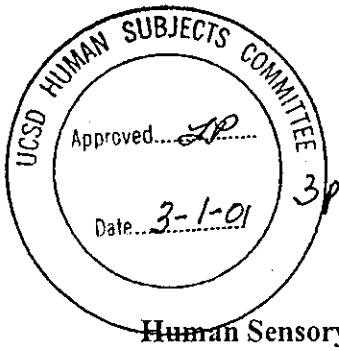
No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

010201



398 **University of California - San Diego**

Consent to Act as a Research Subject

~~Human~~ Sensory Reactions to Chloropicrin: Irritation and Odor (Phase 3b)

William S. Cain, Ph.D., and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age and because you have participated in an earlier phase of this investigation. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

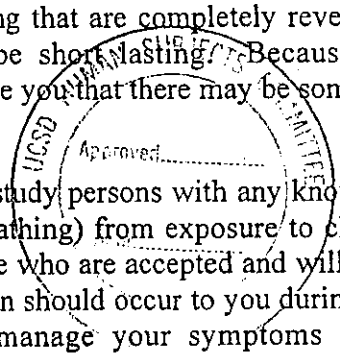
Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, in subsequent sessions we will perform various tests of how your nose and eyes respond to 30-minute exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in two six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all 6 days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15 per hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $900. There will be no direct benefits to you of participation in this research. However, the investigators may

learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.



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you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

Subject's signature	Witness	Date

010201
30 Day

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN SUBJECTS COMMITTEE

Date: April 19, 2001

To: Dr. William S. Cain Mailcode: 0957

Re: Project #010201
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

This letter is to remind you that approval for the above-referenced project has not yet been granted. In order for approval to be granted, the conditions and requirements set forth in the Committee's letter of March 6, 2001, (copy enclosed) must be satisfied within 30 days of today's letter.

If you have any questions about this policy or the contents of our original letter, please call me at x44520.

Sincerely,

Lucille Pearson /KR

Lucille Pearson, Director
Human Subjects Program
0052 X44520

UNIVERSITY OF CALIFORNIA, SAN DIEGO

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Reply to:
Chemosensory Perception Laboratory
University of California , San Diego
La Jolla, CA 92093-0957

Tel: +858-622-5831
Fax: +858-458-9417
e-mail: wcain@ucsd.edu

7 June 2001

To: Ms. Lucille Pearson
Human Subjects Program
MC 0052

From: W. S. Cain WSC

JUN 07 2001

Re: Protocol 010201

I have made the changes requested in points 1-4 of your letter of March 6. I have in addition made some changes requested by the sponsor, the Chloropicrin Manufacturers' Task Force (CMTF). Some of these were made at the request of legal counsel of the CMTF. As far as I can tell, those changes have no substantive impact on the protocol. Nevertheless, there was one additional change made, namely, to increase the number of subjects in phases 1 and 2 of the project from 50 to 60. This came from a statistical consultant hired by CMTF to look at the statistical power of the experiments.

Because I had no control over the timing of the requests from CMTF, but knew that they were coming, I was not able to complete the revisions by May 19, a date you specified in a letter of April 19. I hope that this will not entail going through another submission, but if necessary I will.

Thanks for your assistance.

Title of Project: Human Sensory Irritation Testing for Chloropicrin

Principal Investigator: William S. Cain, Ph.D., Professor of Surgery (Otolaryngology)

Co-Investigators: Alfredo Jalowayski, Ph.D., Research Specialist, Depts. of Pediatrics and Surgery (Otolaryngology), Terence M. Davidson, M.D., Professor of Surgery (Otolaryngology-HNS), Thomas Bruff, M.D., M.P.H., Assistant Clinical Professor of Medicine, Center for Occupational & Environmental Medicine, and Roland Schmidt, Ph.D., Postgraduate Researcher, Dept. of Surgery (Otolaryngology)

1. Facilities: The work will be performed in the Chemosensory Perception Laboratory at the La Jolla Village Professional Center.

2. Duration: One to two years.

3. Specific Aims: There are three phases of work, each with a specific aim, as follows:

Aim 1: To establish the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the sites.

Aim 2: To establish sensitivity for ambient exposures where all channels for detection are available and, within this framework, to establish whether sensitivity varies among exposures of different durations, ranging from a few seconds to 30 minutes. Of particular interest with respect to time-course is whether undetectable concentrations become detectable over time.

Aim 3: To establish whether mildly irritating ambient exposures of one-half hour per day over four days lead to a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue.

Aims 1 and 2 pertain to all persons, those exposed adventitiously from environmental releases of chloropicrin and those exposed occupationally. Aim 3 pertains primarily to persons exposed occupationally and more likely to have repetitive exposure. Aim 3 can also apply to residential exposure because off-gassing may occur for several days from a single field. If there are multiple fields in an area, residents could conceivably be exposed intermittently for a longer time.

4. Background and Significance

Chloropicrin is a colorless liquid with a sharp, penetrating odor. The material is used primarily to fumigate fields in order to control soil-borne fungi, plant diseases, and nematodes. Pre-planting soil fumigation with chloropicrin makes it possible for plants such as strawberries to achieve exceptional root-growth unaffected by soil-pests and

disease. Because of the sharpness of its vapors, chloropicrin has also been added to other, odorless fumigants, such as sulfuryl flouride and methyl bromide as a warning agent. The same property of sharpness has led to use of the material as an agent to test the vapor attenuating properties of activated carbon and the fit of personal protective respiratory devices.

The mammalian acute and chronic toxicology of chloropicrin, including that following inhalation, is well described and current. Testing in laboratory animals by inhalation or other routes of exposure has indicated that chloropicrin in nonsystemically toxic exposures poses no hazard to pregnancy or to the developing fetus, nor does it impair reproductive functioning. It has been found to be noncarcinogenic following lifetime inhalation exposure to rats and mice and in chronic feeding studies to beagle dogs. A chronic oral (gavage) study in rats produced a single animal with a stomach papilloma in the test group receiving 10mg/kg/day, the highest dose tested. This papilloma is considered to be spontaneous in origin. There is no evidence that chloropicrin will bioaccumulate in mammalian cells.

Human beings come into contact with chloropicrin principally on the job in agriculture or, to a lesser extent, in wood preservation. The American Conference of Governmental Industrial Hygienists (ACGIH) initially set the threshold limit value (TLV) at 1 ppm (time-weighted average, TWA) in 1957 and reduced it to 0.1 ppm in 1959, where it remains today. In the documentation, ACGIH states: "A TLV-TWA of 0.1 ppm is recommended for repeated exposure to chloropicrin to prevent eye irritation and the potential for pulmonary changes" (p. 299). OSHA in the U.S. and its regulatory counterparts in many industrial countries also use a TWA of 0.1 ppm as the permissible exposure limit.

The repellent properties of chloropicrin at low levels include reflex blepharospasm, tearing, and pungency that can effectively function as warning properties. As Krieger (1996) noted in an exposure and risk assessment: "This protective reflex response is an important homeostatic mechanism by which exposure is reduced and the sensitive human pulmonary system is spared adverse effects resulting from higher levels of chloropicrin. ...It is a protective reflex response that occurs when chloropicrin contacts the trigeminal nerves of the moist nasal airways. Tearing (lachrymation) and painful stinging eyes results from temporary disturbances of the eye that are completely reversible and occur at concentrations of 0.15 ppm to 0.3 ppm chloropicrin. This undeniable reflex response warns persons to move to uncontaminated environments before toxic exposures occur." (Pp. 10-11.)

Krieger noted further that the mandated use of chloropicrin to warn of the presence of odorless fumigants "gives clear evidence of regulatory recognition of the importance and usefulness of the warning properties of low levels of chloropicrin" (p. 12). To illustrate, U. S. EPA PR Notice 84-5 describes the language to be used for methyl bromide that contains chloropicrin: "This product contains chloropicrin as a warning odorant. Chloropicrin may be irritating to the upper respiratory tract, and even at low levels can cause painful irritation to the eyes, producing tearing. If these symptoms occur, leave the fumigation area immediately." (U. S. EPA, 1984; p. A-1).

Table 1, from Krieger (1996), shows human responses to airborne chloropicrin at various levels and at exposures from instantaneous to chronic to the extent that these have been studied. In the main, the effects derive from direct contact between chloropicrin and tissue at the portal of entry. The nausea and vomiting that may occur from high levels of exposure are thought to occur because of swallowing of the irritating material that has dissolved into saliva.

As detailed below, human responses to airborne chloropicrin are known mainly through anecdotal reports or from studies and other observations collected many decades ago. These experiences indicate that exposure to airborne chloropicrin concentrations of about 0.15ppm to 0.3 ppm can cause tearing and eye irritation that is reversible upon cessation of exposure.

TABLE 1. Human Responses to Airborne Chloropicrin Exposures[1]

Exposures	Conc. x Time	Response	Characteristics
Workplace or Off-Site	Less than TLV-TWA 0.1 ppm or less	None No Adverse effects (eye): NOAEL	None
Instantaneous Contact	0.15 to 0.3 ppm x secs	*Common* Chemical *Sense Threshold*	Tolerable *with very slight or no irritating sensation*
		Reflex tearing and reflex coughing	① Concentration dependent
			②Response ceases when exposure removed
			③Response non-cumulative
			④Primary irritant, little or no systemic effect
	TLV-TWA: 0.1 ppm or less		
Acute	Greater than TLV-TWA of 0.1 ppm; seconds-minutes	Irritation	*Slightly irritating, some stinging or burning of eye, nose, throat*
	0.15-0.3 ppm; 3.30 secs.	Lacrimation	
	0.3-0.37 ppm; 2-2.5 seconds	Eye irritation; LOAEL	
	0.9 ppm	Odor threshold	
	1.5 ppm; few seconds		⑤ Secondary airway irritant; lacrimation, nausea, vomiting
	4 ppm; few seconds	Pulmonary Toxicity	Upper respiratory tract irritation; tissue injury; edema
Chronic	0.1 ppm	No Effect	None

[1]To the best of our knowledge, no controlled human studies of chloropicrin exposure have been completed. Each of these values in this table is anecdotal or derives from a source for which analytical verification of chloropicrin concentrations and standardized evaluation of subject response does dot exist. The present protocol describes a laboratory study that incorporates proper and comprehensive control of variables as well as appropriate analytical and psychophysical response measurement technology to assure valid results having the greatest degree of scientific certainty..

The present investigation concerns specification of the lowest concentrations of chloropicrin detectable by human beings. The investigation will address two situations: 1) the environmental case, where a person typically unconnected with the application of chloropicrin (e.g., a resident or passerby) is exposed, and 2) the occupational case, where exposures may occur episodically over some days. In that case, the work goes beyond specification of mere detection and into the consequences of relatively brief (30 min) repeated exposures at a low level of irritation. Possible consequences of interest include low-level inflammatory reactions of the eyes and upper airways. No animal study can supplant the human tests for precision or relevance. To conduct these studies, human volunteers are to be exposed to chloropicrin vapor and evaluated for ocular irritation, nasal irritation, odor perception (of chloropicrin) and for indications of pulmonary irritation in a laboratory setting in which chloropicrin vapor concentrations and sensory responses can be readily and precisely monitored. Goals of the research are to establish thresholds for these responses, to begin to understand the magnitude of human response variability, if any, to chloropicrin exposure and to improve understanding of differences between animal and human responses to chloropicrin.

The results of these studies will provide an appropriate framework to learn whether odor or sensory irritation that signals low-level exposure to chloropicrin provides an adequate mechanism to prevent occurrence of adverse effects seen in high-dose animal studies. This work also will allow evaluation of important human attributes of the responses to low-level chloropicrin; individual variability in responses; differences in sensitivity as a function of modality of response (ocular stimulation vs, nasal stimulation); and repeated vs. single exposure responses that may be important manifestations of the sensory response to chloropicrin.

Together, the information from these studies will be useful not only in human hazard assessment, but also for the promulgation of exposure standards for workers and the general population.

This research plan was devised in response to a request for proposals from the Chloropicrin Manufacturers Task Force. The protocol was, however, created by the investigators at UCSD.

5. Progress

We have not previously studied this chemical and there appear to be no modern controlled psychophysical studies of it.

6. Research Design and Methods

Phase 1. Measurement of Sensitivity to Momentary Exposures

Objective: To establish in 60 screened subjects (30 males and 30 females) the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the individual sites. Exposures will represent initial perception via the nose and eyes. Relationships of interest will

comprise psychometric (concentration-response) functions for olfactory sensitivity, sensitivity to nasal pungency, and sensitivity of the eyes. Such functions describe the probability of correct detection vs concentration. The highest points on such functions represent consistent detection of the material, not more.

Test Material: Chloropicrin (CCl_3NO_2; CAS #76-06-2) will be the test material throughout the investigation. It is liquid at room temperature (b.p. 112°C). It has a vapor pressure similar to that of water (18.3 mm Hg @ 20°C; 24 mm Hg @ 25°C), has relatively low solubility in water (1.6 g/L @ 25°C), and is miscible in most organic solvents. Chloropicrin will be received in the laboratory in 2-ml quantities in order to insure that in handling of samples no large amounts can spill.

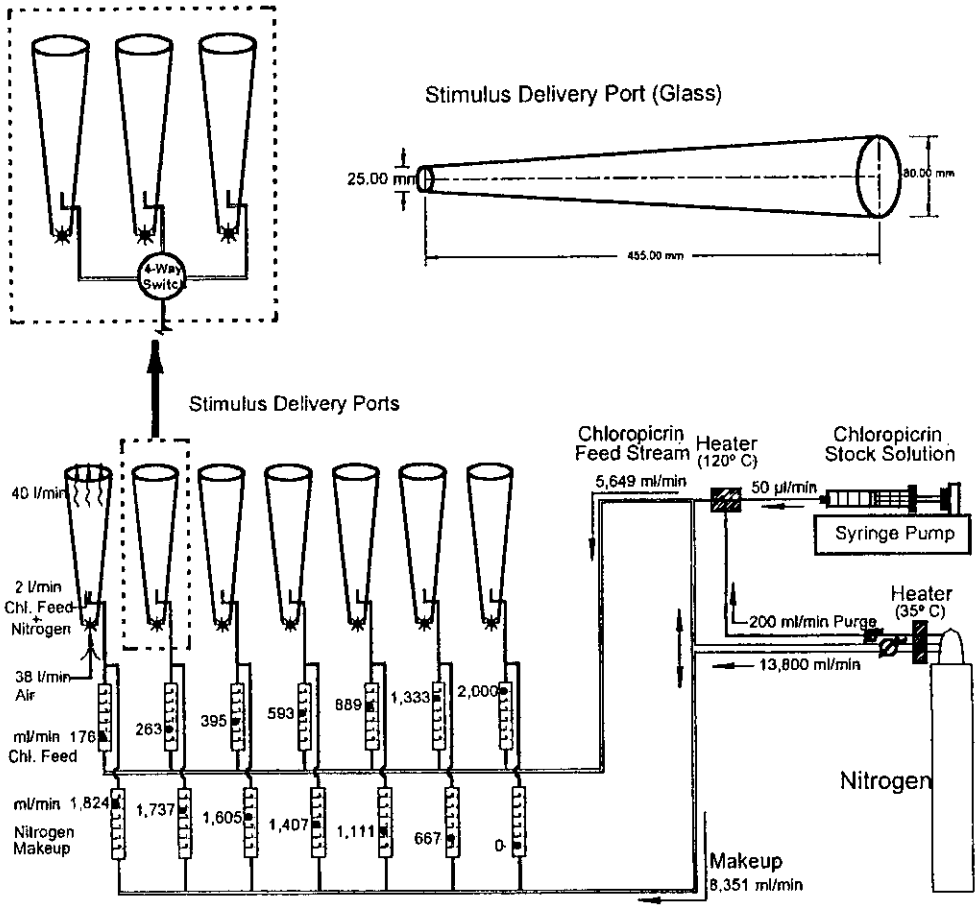


Figure 1. System to determine sensory detection for momentary exposures to chloropicrin vapor. The system will entail splitting a feed stream of chloropicrin-containing nitrogen into seven lines and diluting each with nitrogen and air to achieve the concentration of interest at the end of glass conical delivery ports.

Apparatus: Concentrations of chloropicrin will be presented in Phase 1 by the vapor delivery device (VDD) shown in Fig. 1. It will be placed inside an environmental chamber where ventilation rate will equal 20 air changes per hour. A concentration series will be set up at the beginning of a day and samples taken for calibration at a point shown to be at steady state. The maximum concentration in the series will lie in the vicinity of 0.1 to 0.3 ppm and adjacent steps will differ by a factor of approximately 1.25. Measurement of concentration will be performed via gas chromatography with an electron capture detector (ECD). Calibrations will need to lie within 20% of nominal values for testing to proceed. Calibration will be repeated at appropriate intervals during a day of testing.

Figure 2. Showing three stations of the vapor delivery device. Station #1 delivers the highest test concentration, station #2, the next higher, and so on. In testing, subjects start at the weakest concentration, station #8, and work progressively up to station #1. At each station, the cone of the three with stimulus material, i.e, the active cone, can vary. The inactive cones deliver just background flow. Subjects are blinded to which cones are active.

Procedure: After they have passed screening, subjects will be scheduled for tests with chloropicrin. The subjects will need to agree to participate in four sessions of approximately 5 hours each over a period of weeks. On a day of testing of chloropicrin, six subjects will report to the laboratory approximately 20-30 min before the beginning of testing and will fill out forms to indicate their nasal and ocular health on that day. They will have been told not to eat or drink within the previous 30 min. On the first day of testing for a subject, he/she will receive orientation regarding how to sample from the delivery cones (Fig. 2). He will need to learn how his progress through a series will be signaled with tones to tell him how long to stay in contact with a cone and when to move on to the next cone and the next station.

On any given day, the VDD will be set for an odor series or an irritation series. (We should note that if subjects detect chloropicrin from its feel or irritation at concentrations below its odor threshold, then testing detection via odor will be pointless.) All subjects within the group of six will be making the same judgments, i.e., of odor, of ocular irritation, or of nasal pungency. During testing, subjects will follow one another through the exposures in round-robin fashion.

A given subject will move in progression through the various stations (#8 through #1) of the VDD from low to higher concentrations on a single pass and will make many passes in a session, up to 30. After a pass, he/she will queue up at the end of the group and wait to begin his next round.

At each station of the VDD, the subject will encounter three cones and will need to choose one as containing the test material (three-alternative forced choice). It will take approximately 30 sec to sample the three (five sec per cone, with five sec in-between). After indicating a choice, the subject will move to the next higher level. There will be a 30-sec time-out between the sampling of different levels. For testing of the nose, subjects will wear swimmers' goggles to protect their eyes from the vapor and, for the testing of the eyes, the subjects will wear nose clips. (Pilot work will determine whether subjects are more sensitive to the odor or to the nasal pungency of chloropicrin. Depending on the answer, minor modifications in procedure may be necessary.) A subject will have at least a five-min break between the end of a pass (finishing station #1) and the beginning of another (starting again at station #8), an interval adequate for recovery from any sensory adaptation.

Data analysis: From a subject's various passes through the series of stations in a day, it will be possible to erect a psychometric function. Over his three days of testing with chloropicrin, he/she will produce a function for odor, a function for nasal pungency, and a function for ocular irritation. A function will be accepted into the data set by a criterion of goodness of fit to a theoretical function to be determined from pilot testing. The most likely choice will be a log-normal ogive.

Failure of a function to reach a criterion goodness of fit can indicate lapses of attention on the part of a subject or inability to perform the task. This will be diagnosed within a day because the data will be entered into a spreadsheet for analysis on the same day as a test. This will serve an important role in quality control. A subject who performs unreliably may be given another opportunity, but will be dropped if his/her function fails to show consistency in a second session.

Psychometric functions will be analyzed for their slopes and intercepts, with slope indicative of intra-subject variability and intercept indicative of absolute sensitivity, and ultimately of inter-subject variability. Both the entire functions and certain key parameters, such as the concentrations that lead to 50% correct detection above chance, will be analyzed by ANOVA to inquire about associations of sensitivity with sex, nasal health (as explained below), and age.

Phase 2. Specification of the Sensory Response to Exposures Up to 30 Minutes

Objective: To establish in 60 screened subjects (30 males and 30 females) sensitivity for ambient exposures to chloropicrin where all channels for detection are available and, within this framework, to establish whether sensitivity varies over time for exposures that range from a few seconds to 30 minutes.

Apparatus: The laboratory is equipped with four chambers of dimensions 4' x 8' x 8' (Figs. 3 and 4). Each can hold four seated occupants. The chambers have a single-pass ventilation system that delivers air from a perimeter base-board and exhausts it at the ceiling. Ventilation rate can be varied over a wide range. Testing will take place at normal temperature (22°C) and humidity (RH 40-50%).

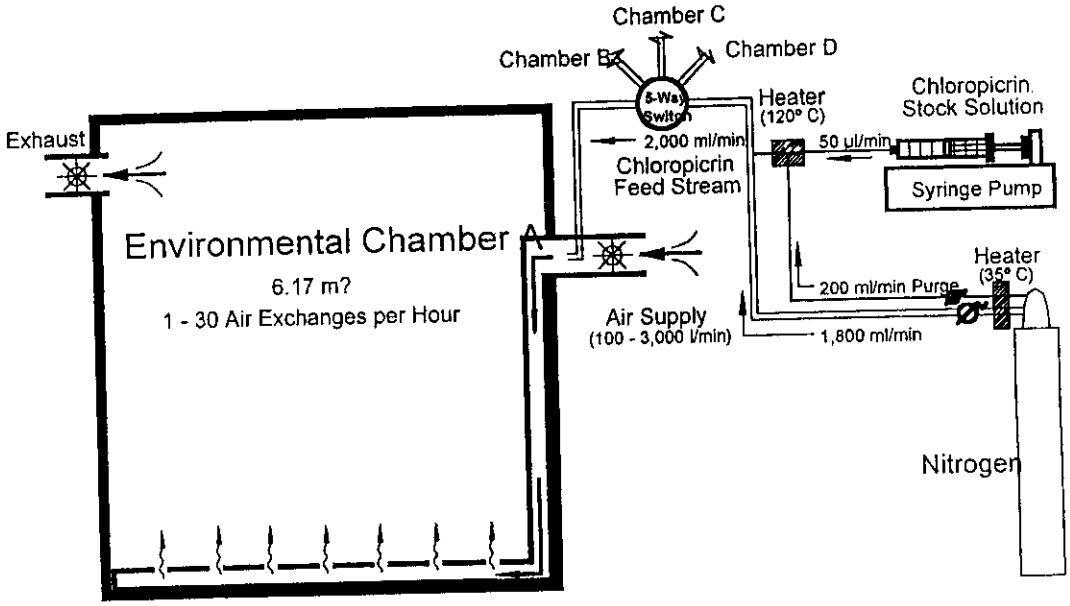


Figure 3. System to deliver controlled amounts of chloropicrin to the environmental chambers.

Levels of chloropicrin will be achieved by the system shown in Fig. 3. Four or five levels will be studied. Assuming that the levels under study exceed the lowest level of detection of the ECD, concentration in the chambers will be measured by direct injection of vapor-samples into the chromatograph (note sampling station in Fig. 4). If the levels do not exceed the lowest level of detection of the ECD, concentration will be monitored by personal sampling pumps placed in the breathing zone of occupants. Samples taken with an adsorbent will be desorbed in the gas chromatograph for analysis.

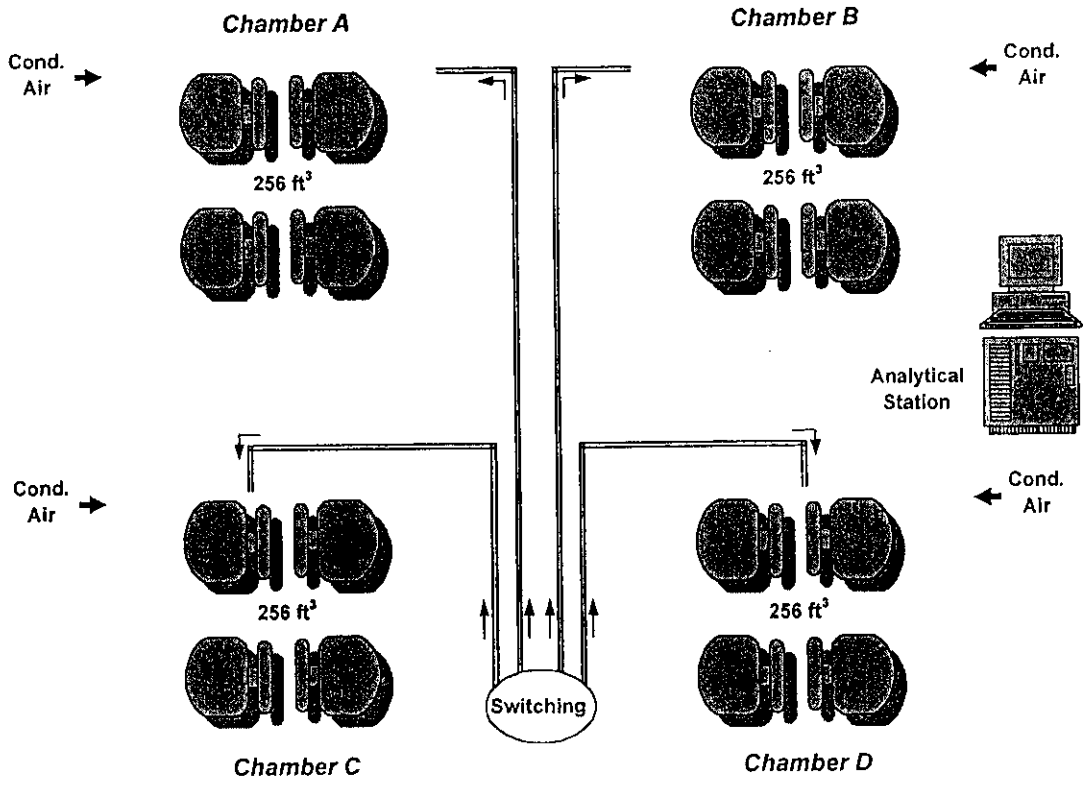


Figure 4. Showing the chambers to be used for exposures in Phases 2 and 3.

Procedure: In brief exposures, subjects will be exposed to the air in the chamber for 5 sec at a time and make a judgment regarding whether or not the chamber appears to contain test material. This will be done with a rating of confidence on a paper ballot: "Is test material present? *Yes* or *No*. Rate confidence from 0 = no confidence, to 5 = high confidence." In addition, subjects will be asked to note through which site(s) they perceived the test material.

For all testing in the chambers, the subject will wear a full respirator covering the mouth, nose and eyes until told to remove it. The reason for the device is that opening and closing the door to a chamber to let in subjects will disturb its atmosphere and subjects will need to be in the environment for a time for the atmosphere to achieve steady state. (Subjects will be fitted with appropriate respirators and taught how to keep them snug. We will test goodness of fit of the respirators in a session before any exposure. It should be borne in mind that the levels will be relatively difficult to detect even without a respirator.) Four subjects facing away from one another can occupy a chamber at once. All four chambers can have four occupants in these circumstances and after a trial has been completed the occupants can move into another chamber and repeat the process. The chamber with the test material can be varied after every fourth exposure and, on that occasion, the concentration changed. This will occur over a series of

concentrations until detection is consistent. (Pilot work will lead to the appropriate range and steps.) The information about which chamber contains the test material and the level of the test material will be unknown to the person who deals with the subjects, i.e., the study will be double blind. We have built a switching device that will feed the chambers appropriate levels of vapor and we will control flow as necessary via a feedback loop.

For the brief duration, subjects will not need to track their sensations while in the chamber, but for the long duration, they will rate irritation periodically throughout the exposure. Nevertheless, the judgment of confidence regarding whether the chamber had the test material will still be the judgment of most interest for it can be compared to whether or not test material was present.

Each subject will be tested at four to five concentrations twice. We anticipate that this can be accomplished in one session per subject for the short duration and two to three sessions for the long duration.

Data Analysis: The outcome of this testing will be the subjects' ratings of confidence in the cases when the test material is present vs. when it is absent. The data can be analyzed via ANOVA for its interval properties, i.e., actual ratings, in order to determine the concentration that can just be discriminated from blank air. The data can also be analyzed for its nominal properties, i.e., correctness vs. incorrectness, to construct receiver operating characteristic (ROC) curves to show level of discrimination. The confidence ratings will be analyzed to test the hypothesis that time alters sensitivity.

Phase 3: Signs/Symptoms of Irritation in Daily Exposures

Objective: To establish whether mildly irritating ambient exposures of one-half hour per day over four days lead to a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The level of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways.

The objective will be addressed in two stages, Phase 3a and Phase 3b.

Phase 3a - Preliminary Study to Validate Techniques of Measurement: Phases 1 and 2 will establish the relative sensitivity of the nose, eyes, and throat to chloropicrin. Phase 2 will establish whether the ability of chloropicrin to provoke irritation cumulates over periods up to a half-hour in ambient exposures. The stimulus-response functions generated in those studies will inform the choice of test concentrations in Phase 3. However, to address Aim 3 we will need additional information obtainable only via a preliminary study. It will serve to establish: a) sensitivity and stability of clinical assessment of signs and symptoms, b) sensitivity and stability of three assays for biochemical markers, and c) sensitivity and stability of the Rhinconjunctivitis Quality of Life Questionnaire in the context of the present work (e.g., Juniper, Thompson, Ferrie, & Roberts, 2000). Statistically speaking, the preliminary study deals with issues of the power to detect effects of exposure to chloropicrin when these exist, but the preliminary

study of Phase 3a will entail no exposure to chloropicrin. It will instead set the stage for exposure to chloropicrin in Phase 3b.

Clinical Studies of Rhinitis: In some respects, the agenda for Phase 3 resembles that of clinical studies of effects of medication on allergic rhinitis. In view of the uniqueness of the present work, the similarities to and differences from those clinical studies seem worthy of comment. In such studies, investigators may challenge subjects with allergens in order to induce reactions and may measure how medication diminishes sensitivity or reactivity. Outcome variables of interest generally include a score for signs/symptoms and perhaps an index of quality of life, such as the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), and some objective indices, such as biochemical markers of inflammation. Variables of this nature have relevance to the present research. In the present case, however, a major question concerns whether subjects will become symptomatic and show signs of rhinitis from environmentally plausible exposures rather than whether they will become less symptomatic from medication. If they become symptomatic, then the outcome variables need to show the result and its various manifestations with clarity. If they do not, then the investigators need to show that the outcome variables would have registered a positive result if it had occurred. This accounts for concerns over sensitivity and stability.

Blinding and a "Reverse Placebo:" The present study differs from clinical trials not only in that the agent of interest may induce symptoms, but also in: a) the inability to blind subjects to presence of the agent, a noxious vapor, b) absence of a reliable way to provoke rhinitis in normals and reluctance to do so needlessly, and c) the time-frame of the effects. In the clinical trials, investigators normally compare subjects treated with the medication, these days typically a topical agent, against those given a placebo. In some instances, such as some studies of seasonal allergic rhinitis, the investigators may not provoke symptoms in the laboratory but may rely upon everyday exposures to keep the subjects symptomatic. In those cases, the investigators gather data before treatment and compare it with data gathered during a regimen of treatment. Even though the studies have the opportunity to use subjects as their own controls, the studies will normally include two groups, one treated with active ingredient and one given placebo. A greater reduction in signs/symptoms in the group treated with active ingredient counts as success (e.g., Van Cauwenberge, Juniper, & the STAR Investigating Group, 2000). In such studies, the subjects who receive the placebo commonly show some subjective improvement, i.e., reduction in symptoms (see Kobayashi, Beaucher, Koepke, Luskin, et al., 1995; Meltzer, Jalowayski, Orgel, & Harris, 1998). If the studies measured effects before vs. after the medication only, the outcome would inflate the benefits of the medication. In the same manner, studies of exposure to an irritating vapor may inflate symptoms unless they use the equivalent of a "placebo." At this point, we can only speculate about whether this will prove true.

Whereas investigators in the clinical trial can blind subjects to the presence or absence of the active ingredient, investigators of a noxious exposure cannot. As an approximation, though, the investigators can expose subjects to a vapor that precipitates no actual irritation, but at least stimulates the same mucosal tissue as an irritating vapor. In the present case, we have chosen a material known as WS 3, a non-irritating, odorless

cooling agent used in consumer products, as this type of "reverse placebo." Its use in Phase 3a will serve to examine the lability of subjects' symptoms as part of the investigation of sensitivity and stability of the ratings of symptoms.

Positive Control Group vs. Positive Control Exposure: Clinical studies provoke symptoms and signs in allergic subjects under conditions well characterized with respect to agent and duration of effect. Hence, when an investigator sprays a dilute solution of ragweed pollen extract into the nose of a person with known allergy to that substance, he can anticipate that an ensuing acute episode may begin within minutes and subside after an hour. The protocol may even entail increasing the concentration of the challenging agent until a response occurs. This establishes the positive control. The study of a noxious vapor cannot easily follow this simple route. It is unclear how to provoke rhinitis in a normal person. An irritating vapor may do so, but at this time we know neither the vapor nor the level that will do so unfailingly. Nor can we know how to provoke the symptoms as temporarily as one might in persons with allergic rhinitis. How then can one demonstrate the sensitivity to resolve the presence vs. the absence of rhinitis in the study of a noxious vapor? One way would be to demonstrate the ability of the outcome measurements to resolve between normals and persons already symptomatic. For this, one can study persons screened for normal nasal health and persons screened for presence of symptomatic allergic rhinitis. By this device, a positive control group substitutes for a positive control exposure.

Ratings: In clinical studies of rhinitis, investigators rather commonly ask subjects to fill out simple ratings of symptoms such as that shown in Table 1 below. The scale reportedly picks up differences of approximately half a step in nasal score with power greater than 90% in groups of a dozen or so subjects, but the published literature contains little documentation of such sensitivity (Meltzer, personal communication).

Although indispensable in clinical studies, ratings of symptoms fail to capture the effects of symptoms on everyday life. This situation has given rise to questionnaires that assess quality of life. The RQLQ, a self-administered questionnaire of high reliability (Cronbach's alpha >0.90) and validity, assesses quality of life as pertains to the nose and eyes. Via a series of 28 questions it assesses how "nose/eye symptoms trouble you in your life." Regarding sleep, for instance, it inquires: "How troubled have you been by each of these sleep problems during the last week as a result of your nose/eye symptoms? 4. Difficulty getting to sleep. [The respondent has six choices per item ranging from *not troubled* to *extremely troubled*] 5. Wake up during night. 6. Lack of a good night's sleep." Most commonly, ratings of quality of life ask the respondent to consider the previous six or seven days. In general, the RQLQ is not given on sequential days, but we see no a prioi reason why it would not work well even though many days of the frame of reference, a week, would overlap.

Clinical studies of rhinitis include ratings from clinicians as well as ratings from subjects. Although the ratings of the clinicians include signs, such as congestion of the nose and tearing of the eyes, they also include the impressions of the subjects. Consequently, it hardly surprises that the ratings of the subjects and the ratings of the clinicians agree extremely well (see Meltzer et al., 1998). In a study that cannot blind the

subject from the nature of an exposure, it seems essential to blind the clinician. The clinician can use a rating scale not unlike that of the subject (Table 2), but should remain oblivious to conditions of exposure to remain unbiased.

Biochemical Markers: Irrespective of whether it arises from allergies, infection, or irritation, rhinitis represents an inflammation of the nasal mucosa. The presence of inflammation reveals itself via the type and number of cells that can be sampled from the mucosa. In samples taken by Rhinoprobe and quantified as number of cells per high power field (HPF), normal superficial nasal mucosa has the following cytologic profile: neutrophils 0-10.5, eosinophils 0-0.45, and basophilic cells 0-0.2 (Jalowayski & Zeiger, 1988). When neutrophils reach 16-20, eosinophils reach 1.1-5.0, and basophilic cells reach 0.4-1.0, subjects characteristically exhibit medically significant inflammation.

The presence of inflammation in mucosal tissue can also reveal itself via levels of biochemical markers. Those shown to increase significantly in nasal and conjunctival mucosa, nasal secretions, and tear fluid after challenge with allergens include albumin, interleukin-8 (IL-8) and soluble intercellular adhesion molecule (sICAM) (Ciprandi, Pronzato, Passalacqua, Ricca, et al., 1996; Calderon, Devalia, Prior, Sapsford, & Davies, 1997; Granstrand, Nylander-French, & Holmstreom, 1998; Wilson, Lau, & Howarth, 1998). Albumin leaks across the mucosal layer during an inflammatory process. Its concentration in nasal fluid and tears collected via absorbent sponge can be measured by ELISA. IL-8, one of several proinflammatory cytokines that play a major role in attracting and activating inflammatory cells, can also be measured by ELISA. sICAM, known to play a role in eosinophil and neutrophil infiltration across endothelial and epithelial cells, can also be measured by ELISA.

The accuracy of research on markers in secretions can depend upon the mode of collection, a constantly evolving matter as investigators abandon error-prone methods. The present research will entail collection of fluid with small cellulose sponges. Such collection does not irritate and avoids uncertainties regarding dilution as in the method of nasal lavage. Weight of the sponge before and after 30-sec application to the mucosa gives an exact measure of amount of fluid collected. The sponge also elutes albumin better than filter paper, another medium of collection. Because collection via the sponge is new, few data address sensitivity to analytes except for eosinophilic cationic protein (ECP) and tryptase in secretions (Klimek, Wolf, Mewes, Dormann, et al., 1999). We believe that we will collect sufficient quantities of nasal and tear fluid to measure levels of albumin, IL-8, and sICAM accurately. The preliminary study will test ability to resolve between normals and symptomatic persons.

Preview of the Preliminary Study: The study described in its particulars below will entail the following: Two groups of subjects, one screened for absence of nasal inflammation and the other for presence of allergic rhinitis will participate in eight half-hour sessions, a block of four on successive days that will entail exposure to air and another block of four on successive days that will entail exposure to a cooling level of WS 3. Half the subjects per category will have the exposure to air first and half the exposure to WS 3 first. In neither case, should the subjects experience irritation from the stimulus. Subjects will have a clinical exam of the nose, eyes, and throat before each

exposure and twice after the exposure, within a half-hour and after another hour. At the approximate times when subjects will have the clinical exams, they will also rate their symptoms. In connection with the clinical exams, the examiner will collect fluid from the nose and eyes for analysis of the markers albumin, IL-8, and sICAM. Before an exposure and 24 hours after the fourth exposure in a block, the subject will fill out the RQLQ with respect to the previous 24 hours.

Questions addressed in the preliminary study will include:

1) How well will a blinded clinical exam resolve between the normals and the subjects with rhinitis? We expect that the exam will resolve between the groups, but the variability day to day, across groups, and across exposures should yield important statistical information about the sensitivity and stability of the exam.

2) How much lability will subjects show in their ratings of symptoms? Will the ratings differ between exposures to air and exposures to cooling agent? The answer may have implications for how to control against biased ratings in studies that cannot blind subjects to exposures. The study will also, however, offer statistical information about the sensitivity and stability of the particular ratings of symptoms (Table 1). How, one might ask, can we know that the cooling agent does not cause irritation and that any increase in symptoms does not occur because of actual irritation? The clinical exam can serve as arbiter here. Moreover, quite possibly the subjects with allergic rhinitis might have their symptoms reduced by exposure to the cooling agent.

3) Will the RQLQ perform meaningfully for resolution between normals and persons with rhinitis when given repeatedly?

4) Will secretions collected with sponges provide enough material for reliable and sensitive assays for the markers albumin, IL-8, and sICAM?

Stated as an objective, Phase 3a entails the following:

Objective: To establish in 32 subjects (16 males and 16 females), half of them (eight males and eight females) screened for normal mucosal condition and the other half for allergic rhinitis, the stability and sensitivity of various tests to reflect the presence of mucosal inflammation.

Table 1

Rating of Symptoms

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Collected by:_____

Use the following scale to indicate the degree of symptoms:

Scale	Degree	Meaning
0	None	No symptom.
1	Mild	Symptom present, but minimal awareness, easily tolerated.
2	Moderate	Symptom definite and bothersome, but tolerated.
3	Severe	Symptom hard to tolerate and can interfere with activities of daily living or sleeping.

Nose

a. Congestion ____
b. Runny Nose ____
c. Itchiness/Sneezing ____
d. Irritation ____ ____

Eye

a. Tearing ____
b. Puffiness ____
c. Itchiness ____
d. Irritation ____ ____

Throat

a. Cough ____
b. Hoarseness ____
c. Dryness ____
d. Irritation ____ ____

Total Score ____

Table 2

Rating of Signs

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Examined by:_____

Use the following scale to indicate the degree of clinical signs:

Scale	Degree
0	No sign evident.
1	Sign barely present.
2	Sign clearly present.
3	Sign quite marked.

Nose
a. Congestion ____
b. Rhinorrhea ____
c. Erythema ____ ____

Eye
a. Tearing ____
b. Puffiness ____
c. Erythema ____ ____

Throat
a. Cough ____
b. Swelling ____
c. Erythema ____ ____

Total Score ____

Apparatus: Exposures will take place in the chambers, with concentration of WS 3 (n-ethyl-5-methyl-2-(1-methylethyl)-cyclohexanecarboxamide, CAS 39711-79-0) controlled in the manner shown for Phase 2, i.e., vaporization of liquid injected onto a warmed surface. Other apparatus will include that used in screening (see details under **7. Human Subjects**).

Procedure: Screening will establish two groups of subjects, 16 with and 16 without evidence of nasal inflammation (see details under **7. Human Subjects**). Every subject will participate in the same tests that will revolve around eight exposures of one half-hour in the chambers. Four exposures will take place in one block of four days and four in another block, with a minimum four-day break between blocks. In one span of four days, subjects will have exposure to WS 3 at a just-cooling level and, in the other span, those same subjects will have exposure to just air on the four successive days. Half the subjects will have exposure to WS 3 first and half exposure to air first.

On each day of exposure, the following will be performed before exposure begins (details of exams appears under **7. Human Subjects**):

1) subject fills out the RQLQ,

2) subject rates symptoms (see Table 1),

3) clinical examination of the nose, eyes, and throat, and rating of signs (seeTable 2),

4) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, and

5) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min.

The following will then be performed in the chamber:

1) subject enters with a full-face respirator in place while vapor concentration is established and removes the respirator on cue to begin half-hour exposure, and

2) in the last half-minute of exposure, the subject rates symptoms.

Fifteen min after exposure, the following will be performed:

1) subject rates symptoms,

2) clinical examination of the nose, eyes, and throat, and rating of signs,

3) sample of nasal secretion taken, as before exposure, and

4) sample of tear fluid taken, as before exposure.

This procedure will be repeated during the four days of exposure in a series. On the day after the fourth exposure of a series, the subject will fill out an RQLQ and return it by mail or phone in the answers.

Data Analysis: Ratings of symptoms (scores per site and total score) will be analyzed for reliability, for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis, and for influence of exposure to WS 3 vs. air.

Score on the ROLQ will be analyzed for reliability, for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis, and for influence of exposure to WS 3 vs. air.

Ratings from the clinical exam will be analyzed similarly as the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions will be assessed for the markers albumin, IL-8, and sICAM. The quantities will be compared for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

As noted at the outset, this study concerns statistical power. From a practical standpoint with respect to Phase 3b, these analyses will boil down to assessment of the number of subjects needed to establish meaningful effects in the study of exposure to chloropicrin.

Phase 3b - Daily Exposures, Signs and Symptoms of Irritation, and Evidence of Inflammation: The work in Phase 3b will build upon that of Phase 3a, but will entail exposure of subjects to chloropicrin.

Objective: To establish in 16 males and 16 females, screened for normal mucosal condition, whether mildly irritating ambient exposures to chloropicrin of one-half hour per day over four days lead to: a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The levels of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways. (The numbers 16 males and 16 females may require modification since results of Phase 3a will determine the number of subjects needed for criterion levels of power.)

Apparatus: Exposures will take place in the chambers. Concentration will be controlled as indicated above in Phase 2. Other apparatus will include that used in screening.

Exposures will entail two concentrations of chloropicrin and a blank. The lower concentration of chloropicrin will equal 0.1 ppm, the occupational threshold limit value (TLV). The higher concentration will equal that detectable on approximately 75% of trials in Phase 1. Most likely, this will occur at about 0.15-0.20 ppm.

Procedure: Subjects will serve in three blocks of six sessions, each beginning on a Friday and ending on a Friday. Exposures to a given concentration in the chambers will last one half-hour on four successive days, Monday through Thursday. In one block, a subject will have exposure to chloropicrin at 0.1 ppm. In another block, the subject will have exposure to a just-irritating level, as indicated above, and, in a third block, the subject will have exposure to just air on the four days. The order in which a given subject has exposure to the three conditions will vary to prevent confounding of order of exposure with level. Except for personnel who set and monitor the conditions in the chamber, the personnel who will deal with the subjects and the subjects themselves will be blinded to the conditions at any given time. At least one week will separate the end of one block and the beginning of another for a subject.

On the first Friday of a block, baseline measures will be taken (see Table 3). The following will be performed:

1) a Rhinoprobe sample taken from the inferior turbinate in one nostril to establish the number and kind of cells in the mucosal layer (see details under **7. Human Subjects**),

2) subject fills out the RQLQ,

3) subject rates symptoms (see Table 1),

4) clinical examination of the nose, eyes, and throat, and rating of signs,

5) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, and

6) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min,

On Monday through Thursday, the following will be performed before the subject enters the chamber:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination of the nose, eyes, and throat, and rating of signs,

4) sample of nasal secretion taken,

5) sample of tear fluid will be taken,

6) office spirometry to establish forced vital capacity (FVC) and forced expiratory volume at 1 sec (FEV_1),

7) exhaled nitric oxide (NO) measured via the mouth while exhaling through an expiratory resistance to indicate NO generated in the lungs, and

8) nasal resistance measured by active, anterior rhinomanometry.

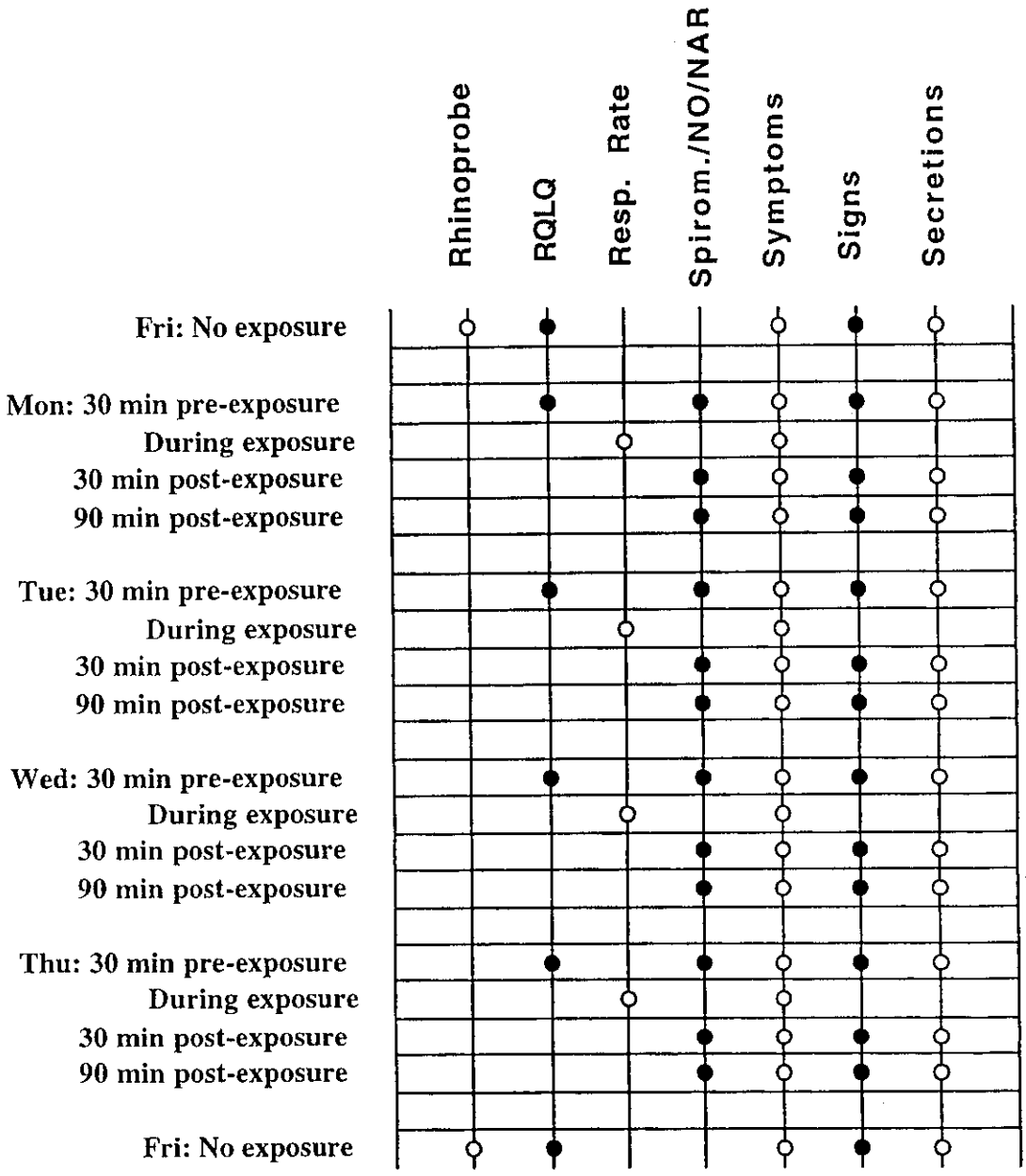
The following will then be performed in the chamber:

1) subject enters with a full-face respirator in place while vapor concentration is established and removes the respirator on cue to begin the half-hour exposure,

2) respiratory rate monitored remotely via Respibands placed around the thorax, and

3) in the last half-minute of exposure, the subject rates symptoms.

Table 3


Rhinoprobe
RQLQ
Resp. Rate
Spirom./NO/NAR
Symptoms
Signs
Secretions
Fri: No exposure
Mon: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Tue: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Wed: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Thu: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Fri: No exposure

Thirty min and 90 min after a session, the following will be performed:

1) subject rates symptoms,

2) clinical examination, with rating of signs,

3) nasal secretion taken, as before the session ,

4) tear fluid taken, as before the session,

5) office spirometry, as before the session,

6) measurement of nasal resistance, as before the session, and

7) exhaled NO measured, as before the session.

On Tuesday, Wednesday, and Thursday, the cycle will be repeated, i.e., a pre-session evaluation and two post-session evaluations.

Approximately 24 hr after the Thursday session, the subject will return and the following performed:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination, with ratings of signs,

4) nasal secretion collected, and

5) Rhinoprobe sample taken from the opposite nostril from the first.

Data Analysis: For interpretation of the study, we will distinguish between variables meant to monitor safety and those of substantive interest.

With respect to safety:

Respiratory rate will be monitored to examine whether the subject remains relaxed during exposures. An unexpected rise can indicate anxiety and gives reason to query the subject about any perceived threat.

Results of the office spirometry will be compared before and after exposure to examine whether a subject has experienced bronchoconstriction from the exposure. A reduction of FEV_1 of 15 % will be reason to remove a subject from further exposures at the level that caused the constriction.

Exhaled nitric oxide (NO) will be compared between after and before to examine whether a subject has developed any inflammation in the lungs.

With respect to substantive interest:

Score on the RQLQ will be analyzed for effects of level of exposure (0 ppm, 0.1 ppm, >0.1 ppm), of day as an indicator of total exposure, and of sex.

Ratings of symptoms (scores per site and total score) will be analyzed for effects of level of exposure, of time since exposure (30 min and 90 min post-exposure), of day as an indicator of total exposure, and of sex.

Ratings of signs on the clinical examination will be analyzed similarly to the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions from both the nose and the eye will be assessed for total mass collected and for the markers albumin, IL-8, and sICAM. The quantities of the biochemical indices will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

Nasal resistance will be compared before vs. after exposure as an aspect of dose of chloropicrin vapor. A blocked nasal passage means lower dose than a patent passage. An increase in resistance is a normal response to inhalation of an irritating vapor. Nasal resistance will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex.

The cells in the Rhinoprobe samples will be compared from the first to the sixth days as an index of cumulative effect of exposure.

7. Human Subjects

Inclusion Criteria for Normal Subjects:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

4) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Normal Subjects:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses. ~~(Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)~~

8) Abuse of alcohol (more than three drinks of alcohol a day over the last year) ~~or drugs~~.

9) ~~Smoking of tobacco or marijuana within the previous year.~~ Use of mood altering drugs within the last year.

10) Use of tobacco or smoking of any substance including marijuana within the at year.

11) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

12) Impaired sense of smell.

13) Pregnancy at the time of the study.

14) Evidence of active infection, rhinitis, pharyngitis, clinically-significant inflammation in the nose or throat, or certain structural abnormalities in these regions.

15) Evidence of conjunctivitis, abnormal redness of the eyes, or abnormalities of the surface of the eyes.

16) Clinically elevated nasal resistance.

17) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the

lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of any of these conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

Sense of Smell: Screening for smell will entail taking a standardized test, e.g., the Connecticut Chemosensory Clinical Research Center [CCCRC] Test for odor threshold and odor identification (Cain, 1989). Subjects will excluded if their olfactory ability falls below normal by the criteria of the test.

Nasal Resistance: The assessment of nasal resistance will occur both in screening and in testing in Phase 3b. Nasal resistance will be measured via a system of computerized anterior rhinomanometry (RHINO; MultiSpiro, San Clemente, CA) that avoids deformation of the nares. The system relies upon an oxygen-type face-mask to monitor flow from one nostril while a tube sealed via a pressure patch (Rhino Diagnostics, Inc., San Diego, CA) monitors pressure at the other nostril.

During testing, the subject breathes normally through the mask through four cycles per nostril. Signals for pressure and flow are digitized and used to calculate resistance at –1.5 cm water column. Subjects with clinically abnormal resistance, defined as >5 cm H_20/ L/sec will be excluded in screening.

Nasal Cytology: Nasal cytology will be used both in screening and in testing in Phase 3b. Subjects who pass the clinical screening examination of the nose, throat, and eyes may still show evidence of inflammation in cytological analysis. In order to obtain cells, the investigator will use a Rhinoprobe, a flexible curette with a 1-mm diam. cup. Sampling with the Rhinoprobe entails a gentle scrape of 3-mm length along the superficial nasal mucosa of the lower turbinate under visual inspection. The procedure causes minor discomfort for an instant.

For analysis, the specimen is gently spread over a small area of a microscope slide, fixed in 95% alcohol, and stained with Wright-Giemsa stain. It is examined under low power (100 x) to determine the adequacy of the specimen and the areas of interest and then graded under high power (1000 x) for cells.

Nasal cytology can reveal various conditions, as follows (see Table 4): 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection, 3) large numbers of eosinophils or basophilic cells (3+ or 4+) provide evidence of inflammation. When used as an outcome variable in testing in Phase 3b, cells will be counted exactly.

Spirometry: Spirometry will be used both in screening and in testing in Phase 3b. Office spirometry testing will conform to the recommendations of the American Thoracic Society. Data to be acquired from this test include the following: FVC, the total volume of gas exhaled after a full inspiration, and FEV_1, the gas volume exhaled in one second by a forced expiration from a full inspiration. The data will allow calculation of the ratio

FEV_1/FVC. Subjects whose pulmonary function fails to lie at or above 75% of predicted FEV_1 or FVC will be excluded.

Ocular Cytology: Ocular cytology will be performed in screening on impression-samples taken from the conjunctival membrane inside the lower eyelid. The lower lid will be sampled with a 3-mm diam. membrane filter placed at the end of a rod. The rod weighs 60 g and suspension of the rod within a short outer cylinder at the moment of contact insures that 60 g will be exerted on the lid. The samples will be analyzed for the presence of cells in the same way as in nasal cytology.

Table 4
Guide for Grading Nasal Cytograms

Quantitative Analysis	Semi-quantitative Analysis	Grade
Epithelial Cells		
N/A	Normal morphology	N
N/A	Abnormal morphology	A
N/A	Ciliocytophthoria	CCP
Eosinophils, neutrophils		
0*	None	0
0.1-1.0*	Occasional cells	0.5+
1.1-5.0*	Few scattered cells or small clumps	1+
5.1-15.0*	Moderate number of cells and larger clumps	2+
15.1-20.0*	Larger clumps of cells which do not cover the entire field	3+
>20*	Large clumps of cells covering the entire field	4+
Basophilic cells		
0*	None	0
0.1-0.3*	Occasional cells	0.5+
0.4-1.0*	Few scattered cells	1+
1.1-3.0*	Moderate number of cells	2+
3.1-6.0*	Many cells easily seen	3+
>6.0*	Large number of cells, as many as 25 per high power field	4+
Bacteria**		
N/A	None seen	0
N/A	Occasional clump	1+
N/A	Moderate number	2+
N/A	Many easily seen	3+
N/A	Large numbers covering the entire field	4+
Goblet cells***		
0	None	0
1-24%	Occasional to few cells	1+
25-49%	Moderate number	2+
50-74%	Many easily seen	3+
75-100%	Large number, may cover entire field	4+

* Mean of cells per 10 high power fields (x1000)
** Note presence of intracellular bacteria
*** Ratio of goblet cells to epithelial cells, expressed as percentage

Persons with significantly elevated levels of PMNs (neutrophils, eosinophils, and basophilic cells) in the conjunctival membrane will be excluded. Persons with small elevations above normal will not.

Pregnancy: Testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded.

Inclusion Criteria for Subjects with Allergic Rhinitis:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must show evidence of allergic rhinitis.

4) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

5) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Subjects with Allergic Rhinitis:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses. ~~(Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)~~

7) History of ocular abnormalities, other than a need for glasses. ~~(Use of contact lenses is not a criterion of exclusion. Subjects who use such lenses will be tested with them in place.)~~

8) Abuse of alcohol (more than three drinks <u>of alcohol a day over the last year</u>) ~~or drugs~~.

9) ~~Smoking of tobacco or marijuana within the previous year.~~ <u>Use of mood altering drugs within the last year.</u>

10) <u>Use of tobacco or smoking of any substance including marijuana within the at year.</u>

11) Daily use of medication, excluding birth control pills.

12) Absent sense of smell.

13) Evidence of active infection or certain structural abnormalities in the upper airways.

14) Pregnancy at the time of the study.

15) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of signs of allergic rhinitis should be present in the patients. Signs of other conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

As part of the examination, the examiner will also establish if there is a history of seasonal or perennial allergic rhinitis with positive skin test to one or more allergens within previous 12 mo. If skin test results are unavailable, a test will be performed at the Nasal Dysfunction Clinic under the supervision of Dr. Terence Davidson. Positive evidence will be a criterion for inclusion. The examiner will also ask about symptoms. A score ≥ 8 for the combined nasal and eye symptoms, with congestion score ≥ 2 for at least three of the five days prior to screening will be a criterion for inclusion.

Sense of Smell: Screening for smell will entail the same testing as described for normals. Subjects will excluded if their olfactory ability falls into the anosmic zone, i.e., if the subject evinces no olfactory ability whatsoever.

Nasal Resistance: The assessment of nasal resistance will entail the same testing as described above. Subjects will be excluded if their resistance lies above 6 H_2O/L/sec.

Nasal Cytology: Nasal cytology will be performed as described above. Subjects will be excluded if they show the following in their cytograms: 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, or 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection.

Spirometry: Screening will entail the same methods as described for normals.

Ocular Cytology: Screening will be performed by the methods described above for normals. Subjects will not be excluded by this test, except for signs of infection, but the results will be compared with those of the nasal cytology for possible stratification of the sample of subjects by presence or absence of inflammatory cells in the eye.

Pregnancy: Testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded.

8. Informed Consent

Subjects will be recruited from the general population. Formal consent will be sought just prior to screening, typically by staff involved in the screening tests. The consent form will contain the relevant facts regarding the reason for the study, the task at hand, any possible adverse effects, and the opportunity to withdraw at any time without penalty. Consent will be written and the subject will receive a copy of the form along with the subject's bill of rights.

9. Therapeutic Alternatives

Not a therapeutic study.

10. Potential Risks

Screening: The screening procedures of direct visualization of the nasal and ocular mucosae and measurement of nasal resistance pose no realistic risks that we can anticipate. Sampling from the nasal mucosa has been performed routinely by Dr. Jalowayski in the Nasal Dysfunction Clinic for many years and entails minimal risk. The impression cytological sampling from the lower lid, also entails minimal risk.

Testing: The risks associated with testing entail sensory irritation of the upper airways or eyes and remotely in these circumstances asthma. We say "remotely" because the exposures will be to perithreshold concentrations with respect to irritation.

11. Risk Management

Monitoring: Subjects will be monitored visually during testing. Testing will stop if the subject finds the test situation uncomfortable and asks to stop. We will instruct subjects to report any respiratory symptoms immediately.

Actions to be taken if an adverse event occurs:

Upper Airway or Ocular Discomfort: Irritation of the nose or eyes should subside soon after exposure. Should effects last longer than expected, the subject will be instructed regarding how to irrigate the affected area with saline solution. An eye irrigation system exists within the laboratory. Furthermore, over-the-counter preparations for such purposes will be available on-site for the subject to use before leaving the lab and to take home. The subject will be informed that if any such irritation or discomfort fails to show progressive improvement during the ensuing hours, then the subject should page Dr. Terence Davidson who will oversee medical management of the symptoms.

Lower airway reactions: Should exposure cause shortness of breath from apparent bronchoconstriction, then an ACLS-certified staff member will initiate an asthma-management protocol. (This person, to be hired, will have training appropriate to obtain ACLS certification. Candidates will be nurses, respiratory therapists, and EMTs.) An emergency kit to treat acute asthma will include: a) a Ventolin inhaler (albuterol USP inhalation aerosol), b) an AeroChamber to be used with the metered dose inhaler, c) a nebulizer and oxygen source, d) a pulse oxymeter, and e) an epinephrine auto-injector (EpiPen) to deliver a 0.3 mg intramuscular dose of epinephrine (1:1000), and d) apparatus for oxygen therapy.

The protocol for management of acute asthma will be the following: a) if symptoms occur, the subject will be offered treatment with Ventolin (one puff held in the lungs for 10 sec., followed by a second puff 30 sec later), b) the pulse oxymeter will be attached to a digit in order to monitor oxygen saturation, c) if the subject seems unable to inhale from the inhaler adequately, then the nebulizer will be used, d) Dr. Thomas Bruff will be paged and informed of the event, and he will decide how the subject should be managed, e) if the subject obtains relief within 15 min. and Dr. Bruff gives no instructions to the contrary, the subject will be asked to remain in the lab for the next half-hour or until he no longer feels short of breath; he will be called at intervals over the next 8-24 hr to inquire about any late reaction, f) if the subject fails to obtain relief, he will be transported to the emergency room at Thornton Hospital, less than 2 miles from the lab. If the subject shows signs of anaphylactic shock, he can be given an injection of epinephrine from the EpiPen and administered humidified oxygen.

Regarding privacy, personal identifiers such as names or Social Security numbers will not be included in any report. Only the investigators, study sponsor and state and federal regulators will have access to the raw data, unless required by a subpoena.

12. Potential Benefits

There will be no benefits to individual subjects.

This research is motivated by the need to develop a definitive set of data relevant to environmental and occupational exposures to chloropicrin. Since the chemical has very low systemic toxicity compared to its rather strong irritating properties, it is irritation that will dictate permissible exposure levels. (The irritation response, as we have noted, largely protects against systemic exposure.) In so far as the present research makes it possible to set more scientifically defensible exposures, then its potential benefit to occupational and public health is considerable.

Approximately 50% of the ACGIH TLV's are based on sensory irritation and yet there have been almost no controlled studies of such. ~~We seem to be entering a new era when~~ Companies and trade groups that exercise product stewardship over commodity chemicals seek quantitative data on human sensory irritation. As product stewards, these entities are often asked to advise users of their products about hazardous properties. Indeed, they must do so in Material Safety Data Sheets. This matter goes beyond ~~merely~~

setting permissible exposure levels into more intrinsic understanding of the perceptual and physiological effects of the chemicals. We see these developments as salutary.

13. Risk-Benefit Ratio: Minor risk, no individual benefits, but benefits to society as indicated just above.

14. Expense to subject: None anticipated.

15. Payment for Participation: Subjects will receive $15 per hour for participation. subjects can receive their payment (cash) at the end of a session or can allow it to accumulate over sessions by mutual agreement. Subjects will not be reimbursed for travel.

16. Privileges/Certifications and Licenses: Dr. Davidson has privileges at UCSD Medical Center. He indicated to the Human Subjects Program in 1999 that sampling from mucosal tissue, the only procedure where we touch the subject, does not require direct medical supervision. Dr. Jalowayski, who has worked with Dr. Davidson in the Nasal Dysfunction Clinic for more than 15 years, developed the sampling and performs it routinely in the clinic. Dr. Jalowayski will do the sampling in this project.

Dr. Thomas Bruff, board certified in internal medicine and in occupational medicine, is attending staff at UCSD Medical Center. He is ACLS-certified.

17. Bibliography:

American Conference of Governmental Industrial Hygienists (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th ed. Cincinnati: ACGIH.

Cain, W. S. (1989). Testing olfaction in a clinical setting. Ear, Nose, and Throat Journal, 68, 316-328.

Calderon, M. A, Devalia, J. L, Prior, A. J, Sapsford, R. J, & Davies, R. (1997). A comparison of cytokine release from epithelial cells cultured from biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis..Journal of Allergy and Clinical Immunology, 99, 65-76.

Ciprandi, G., Buscaglia, S., Pesce, G., Villaggio, B., Bagnasco, M., & Canonica, G.W. (1993). Allergic subjects express intercellular adhesion molecule – 1(ICAM-1 or CD54 on epithelial cells of conjunctiva after allergen challenge. Journal of Allergy and Clinical Immunology, 91(2), 783-792.

Ciprandi, G, Pronzato, C, Passalacqua, G, Ricca, V, Bagnasco, M, Grogen, J, Mela, G. S. & Canonica, G. W. (1996). Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells: An antiallergic activity. Journal of Allergy Clinical Immunology, 98(6), 1088-96.

Granstrand, P., Nylander-French, L., & Holmstreom, M. (1998). Biomarkers of

nasal inflammation in wood-surface coating industry worker. American Journal of Industrial Medicine, 33(4), 392-9.

Jalowayski, A. A. & Zeiger, R.S. (1988). Examination of nasal or conjunctival epithelium specimens. In Manual of Allergy and Immunology, eds. Lawlor, G. J. and Fischer, T.J.; Little, Brown and Co. Boston/Toronto. Pp. 432-434.

Juniper, E. F., Thompson, A. K., Ferrie, P. J., & Roberts, J. N. (2000). Development and validation of the Mini Rhinoconjunctivitis Quality of Life Questionnaire. Clinical and Experimental Allergy, 30, 132-140.

Klimek, L, Wolf, H, Mewes, T, Dormann, D, Reske-Kunz, A, Schnitker, J & Mann, W. (1999). The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophilic cationic protein and tryptase in nasal secretions. Journal of Allergy and Clinical Immunology, 103, 47-53.

Kobayashi, R. H., Beaucher, W. N., Koepke, J. W., Luskin, A. et al. (1995). Triamcinolone acetonide aqueous nasal spray for the treatment of patients with perennial allergic rhinitis: A multicenter, randomized, double-blind, pacebo-controlled study. Clinical Therapeutics, 17, 503-513.

Krieger, R. I. (1996). An assessment of implied worker exposure and risk associated with chloropicrin loading, application, and field tarping activities following application, and implied exposure and risk for off-field concentrations resulting from soil fumigation. Registration document submitted to CAL-EPA Department of Pesticide Regulation, October 1996.

Meltzer. E.O., Jalowayski, A.A., Orgel, H.A., & Harris, A. G. (1998). Subjective and objective assessments in patients with seasonal allergic rhinitis: Effects of therapy with mometasone furoate nasal spray. Journal of Allergy and Clinical Immunology, 102 (1), 39-49.

Polito, A. J. & Proud, D. (1998). Epithelial cells as regulators of airway inflammation. Journal of Allergy and Clinical Immunology, 102(5), 714-718.

Silkoff, P. E., Robbins, R. A., Gaston, B., Lundberg, J. O. N., & Townley, R. G. (2000). Endogenous nitric oxide in allergic airway disease. Journal of Allergy and Clinical Immunology, 105 (3), 438-448.

U. S. Environmental Protection Agency (1984). Notice to Manufacturers, Formulators, Producers and Registrants of Fumigant Products. PR Notice-84-5.

Van Cauwenberge, P., Juniper, E. F., & the STAR Investigating Group (2000) Comparison of the efficacy, safety and quality of life provided by fexofenadine hydrochloride 120 mg, laratadine 10 mg and placebo administered once daily for the treatment of seasonal allergic rhinitis. Clinical and Experimental Allergy, 30, 891-899.

Wagenmann, M., Baroody, F. M., Desrosiers, M., Hubbard, W. S., Ford, S., Lichtenstein, L. M., & Naclerio, R. M. (1996). Unilateral nasal allergen challenge leads to bilateral release of prostaglandin D_2. Clinical and Experimental Allergy, 26, 371-378.

Wilson, S. J, Lau, L., & Howarth, P. H. (1998). Inflammatory mediators in naturally occurring rhinitis. Clinical and Experimental Allergy, 28(2), 220-7.

18. Industry Studies: Supported by the Chloropicrin Manufacturer's Task Force. The Task Force approached the Chemosensory Perception Laboratory about the need for the information and we designed the study. The UCSD Office of Contracts and Grants Administration (Pamela Tiffany) has negotiated a contract with the Task Force. Activation of the contract awaits approval of the human subjects committee. The University will retain all patent rights to the results and is free to publish the work. The PI has no personal agreements (e.g., consultation, confidentiality) with the Chloropicrin Manufacturer's Task Force.

19. Other Funding: None.

20. Cancer Studies: Not applicable.

21. Biological Materials Transfer Agreement: No materials to be transported.

22. Investigational Drug Fact Sheet: No investigational drugs to be used.

23. Conflict of Interest: Form submitted here.

24. Nursing Staff: No impact.

25. Information Service: Will be completed.

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN RESEARCH PROTECTION PROGRAM

Date: March 11, 2002

To: Dr. William S. Cain Mailcode: 0957

Re: Project #010201
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

To avoid any delay in your renewal for this study, the renewal approval letter was sent to you under separate cover. However, please note that in September, 2001, the name and phone number of this office changed. Please ensure that consent forms and other materials as appropriate be revised to reflect this new information:

Human Research Protection Program
(858) 455-5050

Thank you for your assistance in updating your materials related to research studies.

Sincerely,

D. Masys

/mg

Daniel Masys, M.D., Director
Human Research Protection Program
Mailcode 0052 Phone: 858-455-5050
E-mail: hrpp@ucsd.edu

010201

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN RESEARCH PROTECTION PROGRAM

TO: Dr. William S. Cain Mailcode: 0957

RE: Project #010201
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

The above-referenced project was reviewed and approved by one of this institution's Institutional Review Boards in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56), including its relevant Subparts.

Date of IRB review and approval: **March 7, 2002**

D. Masys

/mg

Daniel Masys, M.D., Director
Human Research Protection Program
Mailcode 0052 Phone: 858-455-5050
E-mail: hrpp@ucsd.edu

Note: All Human Subject research conducted at the VA facility and/or utilizing VA/VMRF funds MUST BE APPROVED by the VA Research and Development Committee prior to commencing any research.

IRB PROTOCOL MONITORING FORM

FIRST NOTICE

Project #: 010201

Date: January 16, 2002 YOUR RESPONSE IS DUE NOT LATER THAN: February 21, 2002
To: Dr. William S. Cain Mailcode: 0957
om: HUMAN RESEARCH PROTECTION PROGRAM (HRPP)
ke: Human Sensory Irritation Testing for Chloropicrin

The DHHS, the FDA and the University of California REQUIRE that the IRB conduct continuing review of ongoing research at intervals appropriate to the degree of risk, but not less than once per year. This form constitutes part of this requirement. Please fill out the following progress report **based on subjects studied at this institution except as indicated in questions 2 and 3.**

1. Has the project been activated? Yes ✓ No____ If yes, is it active now? Yes ✓ No

2. How many subjects have been studied to date at UCSD sites? 0 at all sites?

3. What is the expected accrual needed to complete the study at UCSD sites? 60 at all sites?

FEB 19 2002

4. Have changes in the scientific literature or interim experience with this or related studies changed your assessment of potential risks or benefits to study subjects? No If yes, explain in Summary of Results below.

5. How many subjects enrolled on this protocol had: serious and unexpected reactions? N/A deaths unrelated to the protocol? deaths possibly related? ___ deaths probable or definitely related? ___ withdrew before completing the project? or complaints? ___ **Specify nature of complaint(s) in your summary. Include the date AE's were reported to the IRB. (If you have not yet notified the HSC of serious and unexpected, or unusual reactions or deaths, a completed UCSD Research Subject Injury Report must be returned with this form).**

6. Is there a DSMB (Data Safety Monitoring Board) for this study? Yes___ No ✓ If yes, have you **forwarded all** DSMB reports to the IRB? Yes____ No____.

7. Do you plan to make any changes in the project protocol? Yes_____ No ✓

NOTE: ANY MODIFICATIONS IN THE PROTOCOL *NEED TO BE SUBMITTED AS A SEPARATE ITEM PER AMENDMENT FACT SHEET* TO THE IRB AND MUST BE APPROVED BY THE IRB *PRIOR TO INITIATION* EXCEPT WHERE NECESSARY TO ELIMINATE APPARENT IMMEDIATE HAZARD TO THE SUBJECT. (ANY CHANGES IN ANTICIPATED RISKS OR BENEFITS THAT MAY OCCUR AS A RESULT OF CHANGES IN THE PROTOCOL MUST BE INCLUDED).

8. **SUMMARY OF PROGRESS TO DATE. Please attach on a separate sheet,** a page which summarizes 1) Progress in conducting, monitoring, and analyzing the study; 2) Summary of serious and unexpected reactions; 3) Reason for any subject's, voluntary or involuntary, withdrawal from the study; 4) Preliminary results if available; 5) Changes in the scientific knowledge relevant to the conduct of this study; and 6) Adjustments in study design or consent forms; 7) If it is a multicenter trial, any information garnered from other centers that should be reported to the IRB.

9. **A COPY OF THE CURRENT APPROVED INFORMED CONSENT.**

Approval for this project will expire on **March 1, 2002.** The latest date for receipt of this report to avoid expiration is **February 21, 2002.** Failure to meet this deadline will require a complete application for this project.

If you plan to continue this research, approval from the IRB is required. If there have been no changes in the protocol (or if changes have already been approved by the IRB), you may request continued approval in the space below.

Please indicate: RENEW: ✓ **DO NOT RENEW:**_____

WSCain 0957
Signature of Principal Investigator Mail Code

Date: 02-19-02 Phone Number 858-622-5831

RETURN 20 COLLATED SETS OF THE MONITORING FORM, SUMMARY, AND ONE COPY OF THE CURRENT STAMPED CONSENT FORMS TO: HUMAN RESEARCH PROTECTION PROGRAM, 0052

Date: 19 February 2002
To: Human Research Protection Program
From: W. S. Cain
Re: Project #010201, Human Sensory Irritation Testing for Chloropicrin

8. Summary of Progress to Date:

1) The project has been active since October 2001, but human testing has not yet begun. Such testing will begin within the next few days.
2) N/A
3) N/A
4) All results to date have entailed chemical, rather than psychophysical testing.
5) No changes in scientific knowledge that would have an effect on the study.
6) No adjustments.
7) Not a multicenter study.

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor (Phases 1 & 2)

William S. Cain, Ph.D. and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 30 male and 30 female subjects in the study. You will be asked to participate in approximately 5 sessions of up to 5 hours over a period of a few weeks and scheduled at mutual convenience.

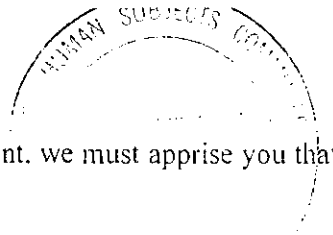
Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to make judgments about whether you experience irritation or odor in brief exposures of your eyes or nose to just air or to very low concentrations of chloropicrin vapor.

You will receive $15 per hour for your time in screening and in testing.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. Because you will be



participating in an experiment. we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

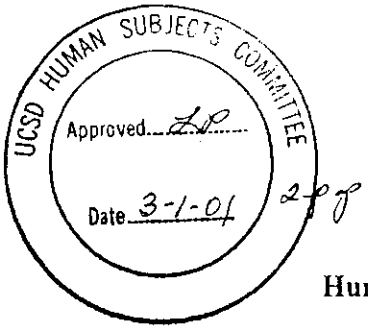
_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date



University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to WS3 (Phase 3a)

William S. Cain, Ph.D. and associates from the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of feel and odor from a chemical called WS3. The chemical is used commonly to impart some sense of feel in personal products. The results are intended to provide information regarding certain testing procedures we will use in later studies on chemical feel.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately 8 sessions of up to 2 hours over a period of a 2-3 weeks.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to WS3, you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by-Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to make judgments about the presence of feel from WS3, to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests.

You will receive $15 per hour for your time in screening and in testing. If you compete all testing, we estimate that you will earn an amount in the vicinity of $300.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

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You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

University of California - San Diego

Consent to Act as a Research Subject

~~Human~~ Sensory Reactions to Chloropicrin: Irritation and Odor (Phase 3b)

William S. Cain, Ph.D., and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age and because you have participated in an earlier phase of this investigation. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, in subsequent sessions we will perform various tests of how your nose and eyes respond to 30-minute exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in two six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all 6 days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15 per hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $900. There will be no direct benefits to you of participation in this research. However, the investigators may

learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (858-616-0857).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 534-4520 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

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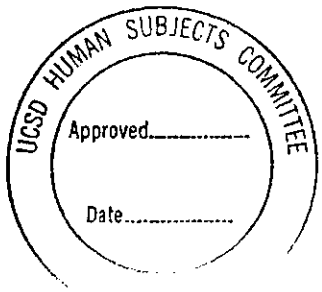
You agree to participate.

Subject's signature	Witness	Date



030246

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN RESEARCH PROTECTIONS PROGRAM

TO: Dr. William Cain Mailcode: 0957

RE: Project #030246
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

The above-referenced project was reviewed and approved by one of this institution's Institutional Review Boards in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56), including its relevant Subparts. This approval, based on the degree of risk, is for 365 days from the date of **IRB review and approval** unless otherwise stated in this letter. The regulations require that continuing review be conducted on or before the 1-year anniversary date of the IRB approval, even though the research activity may not begin until some time after the IRB has given approval.

Date of IRB review and approval: 3/6/2003

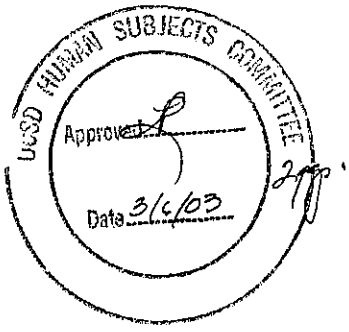
/nm

Daniel Masys, M.D., Director
Human Research Protections Program
Mailcode 0052 Phone: 858-455-5050
E-mail: hrpp@ucsd.edu

Note: All Human Subject research conducted at the VA facility and/or utilizing VA/VMRF funds MUST BE APPROVED by the VA Research and Development Committee prior to commencing any research.

Approval release date: 8/11/2003

030246



University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to a Vapor Blend (Phase 3a)

William S. Cain, Ph.D. and associates from the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of a blend of menthol, camphor, and eucalyptus vapors. The vapor will come from a Sunbeam Waterless Vaporizer. The results are intended to provide information regarding certain testing procedures we will use in later studies on the perception of vapors.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately seven sessions of up to 2.5 hours over a period of two weeks.

Procedures: If you agree to participate, the following will happen. Before you may serve in the main testing with the blend of vapors you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests. We will also take a sample of your nasal secretions by putting a little sponge on the wall between your nostrils and by putting some fluid into your nostrils and asking you to blow it out.

You will receive $15 per hour for your time in screening and in testing. If you complete all testing, we estimate that you will earn an amount in the vicinity of $275.

There will be no direct benefits to you of participation in this research. However, the investigators may learn about how to improve procedures to test biological effects of certain environmental agents not included in the present work.

We will advise you of any significant research findings relevant to your continued participation.

You will experience some discomfort from the testing, such as when we test your breathing, take a scraping from your nose, and take secretions. The discomfort should be mild and brief. We do not expect discomfort from the exposure to vapors. Nevertheless, you will be free to discontinue exposure at any instant. A staff member, who will always

be present, will guide you from the exposure. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, page Dr. Bruff directly at (619) 407-1606).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late or fail to show up for scheduled sessions.–

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call the Human Research Protections Program at (858) 455-5050 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

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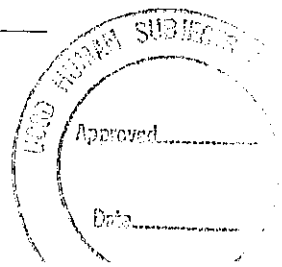
You agree to participate.

_____ _____ _____

Subject's signature Witness Date



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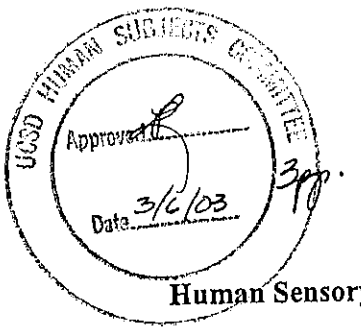

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor (Phase 3b)

William S. Cain, Ph.D., and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. If you are a female and capable of child-bearing, a sample of urine will be collected before the study is begun in order to be as sure as possible that you are not pregnant. Your participation requires that you use a birth control method, such as abstinence, diaphragm, condom or intrauterine device to prevent pregnancy during the study, as chemicals inhaled at irritating levels could possibly harm an unborn child. If you miss a period or think you might be pregnant, you will notify the doctor. You may have to withdraw from the study. If you don't pass screening, you will be excused and paid for your time.

After the screening session, in subsequent sessions we will perform various tests of how your nose and eyes respond to one-hour exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in three six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all 6 days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15 per hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $700. There will be no

direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for health regarding exposure to chloropicrin.

We will advise you of any significant research findings relevant to your continued participation.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. You will be free to discontinue exposure at any instant. If you in the middle of an exposure and wish to stop, a staff member will guide you out of it. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

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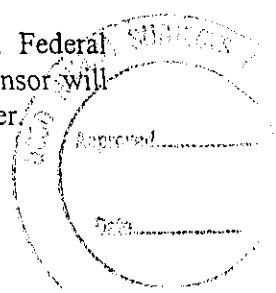

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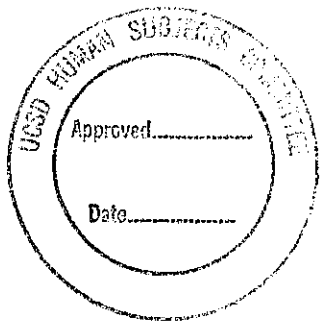


030246

Title of Project: Human Sensory Irritation Testing for Chloropicrin

Principal Investigator: William S. Cain, Ph.D., Professor of Surgery (Otolaryngology)

Co-Investigators: Alfredo Jalowayski, Ph.D., Research Specialist, Depts. of Pediatrics and Surgery (Otolaryngology), Terence M. Davidson, M.D., Professor of Surgery (Otolaryngology-HNS), Thomas Bruff, M.D., M.P.H., Assistant Clinical Professor of Medicine, and Roland Schmidt, Ph.D., Project Scientist, Dept. of Surgery (Otolaryngology)

1. Facilities: The work will be performed in the Chemosensory Perception Laboratory at the La Jolla Village Professional Center.

2. Duration: One to two years.

3. Specific Aims: There are three phases of work, each with a specific aim, as follows:

Aim 1: To establish the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the sites. **(Completed.)**

Aim 2: To establish sensitivity for ambient exposures where all channels for detection are available and, within this framework, to establish whether sensitivity varies among exposures of different durations, ranging from a few seconds to 30 minutes. Of particular interest with respect to time-course is whether undetectable concentrations become detectable over time. **(Completed.)**

Aim 3: To establish whether mildly irritating ambient exposures of one-hour per day over four days lead to a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. **(This aim is the subject of the current IRB application.)**

Aims 1 and 2 pertained to all persons, those exposed adventitiously from environmental releases of chloropicrin and those exposed occupationally. Aim 3 pertains primarily to persons exposed occupationally and more likely to have repetitive exposure. Aim 3 can also apply to residential exposure because off-gassing may occur for several days from a single field. If there are multiple fields in an area, residents could conceivably be exposed intermittently for a longer time.

4. Background and Significance

Chloropicrin is a colorless liquid with a sharp, penetrating odor. The material is used primarily to fumigate fields in order to control soil-borne fungi, plant diseases, and nematodes. Pre-planting soil fumigation with chloropicrin makes it possible for plants such as strawberries to achieve exceptional root-growth unaffected by soil-pests and disease. Because of the sharpness of its vapors, chloropicrin has also been added to

other, odorless fumigants, such as sulfuryl flouride and methyl bromide as a warning agent. The same property of sharpness has led to use of the material as an agent to test the vapor attenuating properties of activated carbon and the fit of personal protective respiratory devices.

The mammalian acute and chronic toxicology of chloropicrin, including that following inhalation, is well described and current. Testing in laboratory animals by inhalation or other routes of exposure has indicated that chloropicrin in nonsystemically toxic exposures poses no hazard to pregnancy or to the developing fetus, nor does it impair reproductive functioning. It has been found to be noncarcinogenic following lifetime inhalation exposure to rats and mice and in chronic feeding studies to beagle dogs. A chronic oral (gavage) study in rats produced a single animal with a stomach papilloma in the test group receiving 10mg/kg/day, the highest dose tested. This papilloma is considered to be spontaneous in origin. There is no evidence that chloropicrin will bioaccumulate in mammalian cells.

Human beings come into contact with chloropicrin principally on the job in agriculture or, to a lesser extent, in wood preservation. The American Conference of Governmental Industrial Hygienists (ACGIH) initially set the threshold limit value (TLV) at 1 ppm (time-weighted average, TWA) in 1957 and reduced it to 0.1 ppm in 1959, where it remains today. In the documentation, ACGIH states: "A TLV-TWA of 0.1 ppm is recommended for repeated exposure to chloropicrin to prevent eye irritation and the potential for pulmonary changes" (p. 299). OSHA in the U.S. and its regulatory counterparts in many industrial countries also use a TWA of 0.1 ppm as the permissible exposure limit.

The repellent properties of chloropicrin at low levels include reflex blepharospasm, tearing, and pungency that can effectively function as warning properties. As Krieger (1996) noted in an exposure and risk assessment: "This protective reflex response is an important homeostatic mechanism by which exposure is reduced and the sensitive human pulmonary system is spared adverse effects resulting from higher levels of chloropicrin. ...It is a protective reflex response that occurs when chloropicrin contacts the trigeminal nerves of the moist nasal airways. Tearing (lachrymation) and painful stinging eyes results from temporary disturbances of the eye that are completely reversible and occur at concentrations of 0.15 ppm to 0.3 ppm chloropicrin. This undeniable reflex response warns persons to move to uncontaminated environments before toxic exposures occur." (Pp. 10-11.)

Krieger noted further that the mandated use of chloropicrin to warn of the presence of odorless fumigants "gives clear evidence of regulatory recognition of the importance and usefulness of the warning properties of low levels of chloropicrin" (p. 12). To illustrate, U. S. EPA PR Notice 84-5 describes the language to be used for methyl bromide that contains chloropicrin: "This product contains chloropicrin as a warning odorant. Chloropicrin may be irritating to the upper respiratory tract, and even at low levels can cause painful irritation to the eyes, producing tearing. If these symptoms occur, leave the fumigation area immediately." (U. S. EPA, 1984; p. A-1).

The table below, from Krieger (1996), shows human responses to airborne chloropicrin at various levels and at exposures from instantaneous to chronic to the extent that these have been studied. In the main, the effects derive from direct contact between chloropicrin and tissue at the portal of entry. The nausea and vomiting that may occur from high levels of exposure are thought to occur because of swallowing of the irritating material that has dissolved into saliva.

Until the present investigation, human responses to airborne chloropicrin have been known mainly through anecdotal reports or from studies and other observations collected many decades ago. These experiences indicated that exposure to airborne chloropicrin concentrations of about 0.15 ppm to 0.3 ppm can cause tearing and eye irritation that is reversible upon cessation of exposure.

The results of these studies will provide an appropriate framework to learn whether odor or sensory irritation that signals low-level exposure to chloropicrin provides an adequate mechanism to prevent occurrence of adverse effects seen in high-dose animal studies. This work also will allow evaluation of important human attributes of the responses to low-level chloropicrin; individual variability in responses; differences in sensitivity as a function of modality of response (ocular stimulation vs, nasal stimulation); and repeated vs. single exposure responses that may be important manifestations of the sensory response to chloropicrin.

Together, the information from these studies will be useful not only in human hazard assessment, but also for the promulgation of exposure standards for workers and the general population.

This research plan was devised in response to a request for proposals from the Chloropicrin Manufacturers Task Force. The protocol was, however, created by the investigators at UCSD.

Human Responses to Airborne Chloropicrin Exposures[1]

Exposures	Conc. x Time	Response	Characteristics
Workplace or Off-Site	Less than TLV-TWA 0.1 ppm or less	None No Adverse effects (eye): NOAEL	None
Instantaneous Contact	0.15 to 0.3 ppm x secs	*Common* Chemical *Sense Threshold*	Tolerable *with very slight or no irritating sensation*
		Reflex tearing and reflex coughing	① Concentration dependent
			②Response ceases when exposure removed
			③Response non-cumulative
			④Primary irritant, little or no systemic effect
	TLV-TWA: 0.1 ppm or less		
Acute	Greater than TLV-TWA of 0.1 ppm; seconds-minutes	Irritation	*Slightly irritating, some stinging or burning of eye, nose, throat*
	0.15-0.3 ppm; 3.30 secs.	Lacrimation	
	0.3-0.37 ppm; 2-2.5 seconds	Eye irritation; LOAEL	
	0.9 ppm	Odor threshold	
	1.5 ppm; few seconds		⑤ Secondary airway irritant; lacrimation, nausea, vomiting
	4 ppm; few seconds	Pulmonary Toxicity	Upper respiratory tract irritation; tissue injury; edema
Chronic	0.1 ppm	No Effect	None

[1]To the best of our knowledge, no controlled human studies of chloropicrin exposure have been completed. Each of these values in this table is anecdotal or derives from a source for which analytical verification of chloropicrin concentrations and standardized evaluation of subject response does dot exist. The present protocol describes a laboratory study that incorporates proper and comprehensive control of variables as well as appropriate analytical and psychophysical response measurement technology to assure valid results having the greatest degree of scientific certainty..

5. Progress

We have collected the data regarding aims 1 (Phase 1) and 2 (Phase 2), but have not yet finished the analysis. The total number of participants who passed screening equaled 126, half males and half females. Almost all of these persons participated in exposures in Phase 1, Phase 2, or both. A small number of people did not go on to either phase, an occurrence we see in every study that entails some time between screening and further participation.

No adverse events were reported in Phases 1 or 2. Subjects tolerated the exposures and no subject terminated because of an inability to tolerate chloropicrin. We believe this reflects our focus on just-detectable stimulation.

As we did note in a file submitted with the application for renewal, one subject who developed a cold before his one day of exposure in Phase 1 speculated that his exposure to chloropicrin may have exacerbated the cold. He raised this issue because his cold was more severe than that of his roommate from whom he thought he caught the cold. The cold resolved in two weeks. This occurred at the end of the school year and the student left the area, so the question of whether he and we might have agreed on his further participation never came up. Since the protocol had specified that we would not run subjects who had "acute illness in the previous month" and since this subject with the beginning symptoms of the cold had sneaked through, not at screening, but at the day of testing, we realized the need to be more vigilant. We need to note, however, that we had no reason to believe that the testing would harm a person with a common cold. Since that event, any subject who indicates more than minimal symptoms before testing has a further interview with a doctoral level professional who goes into details and decides whether the subject may continue. Since that time we have postponed testing three persons until symptoms possibly indicative of a cold have resolved. Testing has then proceeded normally.

Phase 1 focused upon determination of the threshold for odor, for feel in the eyes, and feel in the nose (see Figure 1 for the subject's task of choosing which of three cones at a station had odor). The threshold for odor equaled 750 ppb, averaged over the 63 participants in this phase. The threshold for feel in the eyes equaled 929 ppb (30-sec exposure). And, the threshold for feel in the nose proved indeterminate over the range of concentrations explored. It was therefore clear from the testing that for the average subject, the eyes were more sensitive. The threshold for odor proved to be a little lower than previous estimates and the threshold for feel in the eyes proved to be somewhat higher. Nevertheless, as Phase 2 indicated, the threshold for feel in the eyes is quite time-dependent. We obtained enough data from each subject to make meaningful comparisons of the individual differences that underlie the average threshold. This will be an important part of our analysis over the next months.

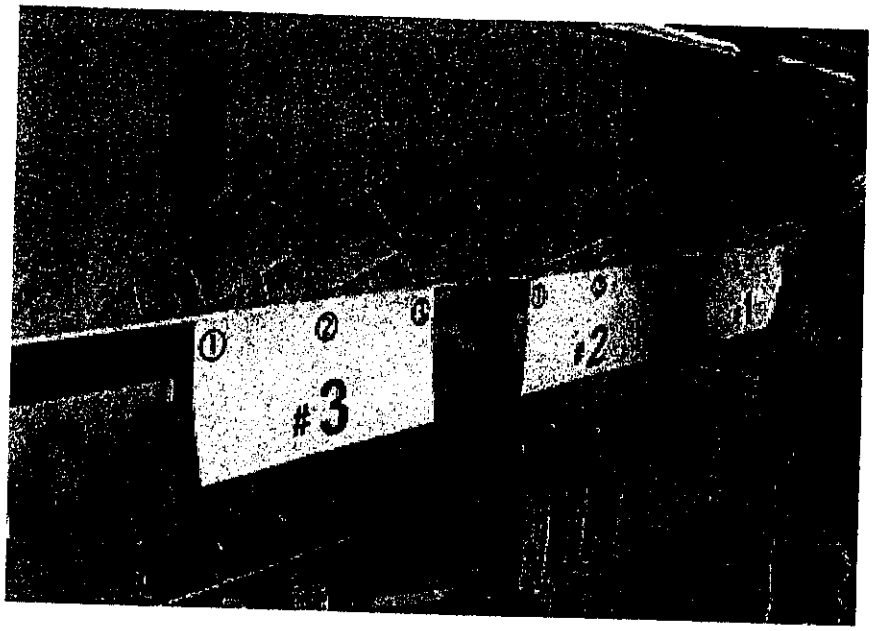


Figure 1. Showing a subject interacting with the delivery device used to present vapors for short exposures (2 sec for odor and nasal feel; 30 sec for feel in the eyes). A computer controlled voice guides the subject through the task.

In Phase 2, a total of 62 subjects sought to detect the presence of feel from chloropicrin in the environment of a chamber and to rate confidence in their judgments of detect-no detect (see Fig. 2). In some exposures chloropicrin was present and in some it was not. (The levels were too low for the subjects to detect any odor.) In this phase we found that over time subjects could detect feel in the eyes at much lower concentrations than the 929 ppb felt in Phase 1. Subjects could, for example, eventually feel a concentration of 75 ppb after many minutes of exposure. Demonstration of the time-dependence of detection of irritation is important new information regarding chloropicrin. As in the testing in Phase 1, individual differences in sensitivity will be a focus of analysis.

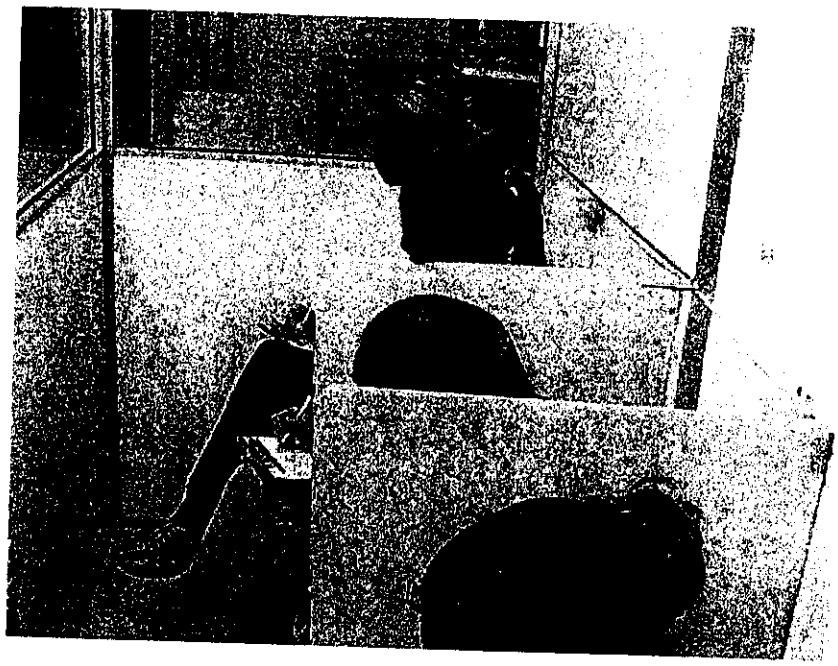


Figure 2. Subjects in the setting for Phase 2, where they sought to detect presence of chloropicrin over time. They made judgments every minute for 20-30 minute sessions. This setting will also be used for the experiments of Phase 3.

As we analyze the results of Phases 1 and 2, we are prepared to move into Phase 3.

Phase 3: Signs/Symptoms of Irritation in Daily Exposures

Objective: To establish whether mildly irritating ambient exposures of one hour per day over four days lead to: a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The level of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways.

The objective will be addressed in two stages, Phase 3a and Phase 3b.

Phase 3a - Preliminary Study to Validate Techniques of Measurement: This phase will serve to establish: a) sensitivity and stability of clinical assessment of signs and symptoms, b) sensitivity and stability of three assays for biochemical markers, and c) sensitivity and stability of the Rhinconjunctivitis Quality of Life Questionnaire in the context of the present work (e.g., Juniper, Thompson, Ferrie, & Roberts, 2000). Statistically speaking, the preliminary study deals with issues of the power to detect effects of exposure to chloropicrin when these exist, but the preliminary study of Phase 3a will entail no exposure to chloropicrin. It will instead set the stage for exposures to chloropicrin in Phase 3b.

Clinical Studies of Rhinitis: In some respects, the agenda for Phase 3 resembles that of clinical studies of effects of medication on allergic rhinitis. In view of the

uniqueness of the present work, the similarities to and differences from those clinical studies seem worthy of comment. In such studies, investigators may challenge subjects with allergens in order to induce reactions and may measure how medication diminishes sensitivity or reactivity. Outcome variables of interest generally include a score for signs/symptoms and perhaps an index of quality of life, such as the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), and some objective indices, such as biochemical markers of inflammation. Variables of this nature have relevance to the present research. In the present case, however, a major question concerns whether subjects will become symptomatic and show signs of rhinitis from environmentally plausible exposures rather than whether they will become less symptomatic from medication. If they become symptomatic, then the outcome variables need to show the result and its various manifestations with clarity. If they do not, then the investigators need to show that the outcome variables would have registered a positive result if it had occurred. This accounts for concerns over sensitivity and stability.

Positive Control Group vs. Positive Control Exposure: Clinical studies provoke symptoms and signs in allergic subjects under conditions well characterized with respect to agent and duration of effect. Hence, when an investigator sprays a dilute solution of ragweed pollen extract into the nose of a person with known allergy to that substance, he can anticipate that an ensuing acute episode may begin within minutes and subside after an hour. The protocol may even entail increasing the concentration of the challenging agent until a response occurs. This establishes the positive control. The study of a noxious vapor cannot easily follow this simple route. It is unclear how to provoke rhinitis in a normal person. An irritating vapor may do so, but at this time we know neither the vapor nor the level that will do so unfailingly. Nor can we know how to provoke the symptoms as temporarily as one might in persons with allergic rhinitis. How then can one demonstrate the sensitivity to resolve the presence vs. the absence of rhinitis in the study of a noxious vapor? One way would be to demonstrate the ability of the outcome measurements to resolve between normals and persons already symptomatic. For this, one can study persons screened for normal nasal health and persons screened for presence of symptomatic allergic rhinitis. By this device, a positive control group substitutes for a positive control exposure.

Ratings: In clinical studies of rhinitis, investigators rather commonly ask subjects to fill out simple ratings of symptoms such as that shown in Table 1 below. The scale reportedly picks up differences of approximately half a step in nasal score with power greater than 90% in groups of a dozen or so subjects, but the published literature contains little documentation of such sensitivity (Meltzer, personal communication).

Although indispensable in clinical studies, ratings of symptoms fail to capture the effects of symptoms on everyday life. This situation has given rise to questionnaires that assess quality of life. The RQLQ, a self-administered questionnaire of high reliability (Cronbach's alpha >0.90) and validity, assesses quality of life as pertains to the nose and eyes. Via a series of 28 questions it assesses how "nose/eye symptoms trouble you in your life." Regarding sleep, for instance, it inquires: "How troubled have you been by each of these sleep problems during the last week as a result of your nose/eye symptoms? 4. Difficulty getting to sleep. [The respondent has six choices per item ranging from *not*

troubled to *extremely troubled*] 5. Wake up during night. 6. Lack of a good night's sleep." Most commonly, ratings of quality of life ask the respondent to consider the previous six or seven days. In general, the RQLQ is not given on sequential days, but we see no a prioi reason why it would not work well even though many days of the frame of reference, a week, would overlap.

Clinical studies of rhinitis include ratings from clinicians as well as ratings from subjects. Although the ratings of the clinicians include signs, such as congestion of the nose and tearing of the eyes, they also include the impressions of the subjects. Consequently, it hardly surprises that the ratings of the subjects and the ratings of the clinicians agree extremely well (see Meltzer et al., 1998). In a study that cannot blind the subject from the nature of an exposure, it seems essential to blind the clinician. The clinician can use a rating scale not unlike that of the subject, but should remain oblivious to conditions of exposure to remain unbiased.

Biochemical Markers: Irrespective of whether it arises from allergies, infection, or irritation, rhinitis represents an inflammation of the nasal mucosa. The presence of inflammation reveals itself via the type and number of cells that can be sampled from the mucosa. In samples taken by Rhinoprobe and quantified as number of cells per high power field (HPF), normal superficial nasal mucosa has the following cytologic profile: neutrophils 0-10.5, eosinophils 0-0.45, and basophilic cells 0-0.2 (Jalowayski & Zeiger, 1988). When neutrophils reach 16-20, eosinophils reach 1.1-5.0, and basophilic cells reach 0.4-1.0, subjects characteristically exhibit medically significant inflammation.

The presence of inflammation in mucosal tissue can also reveal itself via levels of biochemical markers. Those shown to increase significantly in nasal and conjunctival mucosa, nasal secretions, and tear fluid after challenge with allergens include albumin, interleukin-8 (IL-8) and soluble intercellular adhesion molecule (sICAM) (Ciprandi, Pronzato, Passalacqua, Ricca, et al., 1996; Calderon, Devalia, Prior, Sapsford, & Davies, 1997; Granstrand, Nylander-French, & Holmstreom, 1998; Wilson, Lau, & Howarth, 1998). Albumin leaks across the mucosal layer during an inflammatory process. Its concentration in nasal fluid and tears collected via absorbent sponge can be measured by ELISA. IL-8, one of several proinflammatory cytokines that play a major role in attracting and activating inflammatory cells, can also be measured by ELISA. sICAM, known to play a role in eosinophil and neutrophil infiltration across endothelial and epithelial cells, can also be measured by ELISA.

The accuracy of research on markers in secretions can depend upon the mode of collection, a constantly evolving matter as investigators abandon error-prone methods. The present research will entail collection of fluid with small cellulose sponges. Such collection does not irritate and avoids uncertainties regarding dilution as in the method of nasal lavage. Weight of the sponge before and after 30-sec application to the mucosa gives an exact measure of amount of fluid collected. The sponge also elutes albumin better than filter paper, another medium of collection. Because collection via the sponge is new, few data address sensitivity to analytes except for eosinophilic cationic protein (ECP) and tryptase in secretions (Klimek, Wolf, Mewes, Dormann, et al., 1999; Riechelmann, Deutschle, Friemel, et al., 2003). We believe that we will collect sufficient

quantities of nasal and tear fluid to measure levels of albumin, IL-8, and sICAM accurately. The preliminary study will test ability to resolve between normals and symptomatic persons. In order to assess the sensitivity of our assay for samples taken via sponge, however, we must also include use of a nasal lavage. Its use will lie under the rubric of secretions, as indicated in Table 3.

Preview of the Preliminary Study: The study described in its particulars below will entail the following: Two groups of subjects, one screened for absence of nasal inflammation and the other for presence of allergic rhinitis will participate in six sessions of testing on contiguous workdays, with each session approximately 2.5 hours long. On days two through five, the subjects will have one-hour exposures to vapor from the scent pads (essential oils of menthol, camphor, and eucalyptus) of a Sunbeam Health at Home Vaporizer. The product literature describes the vapor as "a blend of soothing essential oils." Furthermore, we have been in touch with the consumer division of the Sunbeam Corp. and they have stated that the ingredients are natural, and nontoxic to the best of their knowledge. Subjects should experience no irritation. Subjects will have a clinical exam of the nose, eyes, and throat before each exposure and twice after the exposure, within a half-hour and after another hour. At the approximate times when subjects will have the clinical exams, they will also rate their symptoms. In connection with the clinical exams, the examiner will collect fluid from the nose and eyes for analysis of the markers albumin, IL-8, and sICAM. Before an exposure and 24 hours after the fourth exposure in a block, the subject will fill out the RQLQ with respect to the previous 24 hours.

Questions addressed in the preliminary study will include:

1) How well will a blinded clinical exam resolve between the normals and the subjects with rhinitis? We expect that the exam will resolve between the groups, but the variability day to day, across groups, and across exposures should yield important statistical information about the sensitivity and stability of the exam.

2) Will the RQLQ perform meaningfully for resolution between normals and persons with rhinitis when given repeatedly?

3) Will secretions collected with sponges provide enough material for reliable and sensitive assays for the markers albumin, IL-8, and sICAM?

Stated as an objective, Phase 3a entails the following:

Objective: To establish in 32 subjects (16 males and 16 females), half of them (eight males and eight females) screened for normal mucosal condition and the other half for allergic rhinitis, the stability and sensitivity of various tests to reflect the presence of mucosal inflammation.

Table 1

Rating of Symptoms

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Collected by:_____

Use the following scale to indicate the degree of symptoms:

Scale	Degree	Meaning
0	None	No symptom.
1	Mild	Symptom present, but minimal awareness, easily tolerated.
2	Moderate	Symptom definite and bothersome, but tolerated.
3	Severe	Symptom hard to tolerate and can interfere with activities of daily living or sleeping.

Nose		
a. Congestion	____	
b. Runny Nose	____	
c. Itchiness/Sneezing	____	
d. Irritation	____	___
Eye		
a. Tearing	____	
b. Puffiness	____	
c. Itchiness	____	
d. Irritation	____	____
Throat		
a. Cough	____	
b. Hoarseness	____	
c. Dryness	____	
d. Irritation	____	____
	Total Score	____

Table 2

Rating of Signs

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Examined by:_____

Use the following scale to indicate the degree of clinical signs:

Scale	Degree
0	No sign evident.
1	Sign barely present.
2	Sign clearly present.
3	Sign quite marked.

Nose
a. Congestion ____
b. Rhinorrhea ____
c. Erythema ____ ____

Eye
a. Tearing ____
b. Puffiness ____
c. Erythema ____ ____

Throat
a. Cough ____
b. Swelling ____
c. Erythema ____ ____

Total Score ____

Apparatus: Exposures will take place in the chambers, with the Sunbeam Waterless Vaporizer operating during the exposures. The vaporizer causes no change in ambient temperature or humidity. Its small heater merely promotes evaporation from a 1" x 2" scent pad. Other apparatus will include that used in screening (see details under **7. Human Subjects**).

Procedure: Screening will establish two groups of subjects, 16 with and 16 without evidence of nasal inflammation (see details under **7. Human Subjects**). Every subject will participate in the same tests that will revolve around four exposures of one-hour in the chambers.

On each day of exposure, the following will be performed before exposure begins (details of exams appears under **7. Human Subjects**):

1) subject fills out the RQLQ,

2) subject rates symptoms (see Table 1),

3) clinical examination of the nose, eyes, and throat, and rating of signs (see Table 2),

4) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, followed by nasal lavage with 10 ml of lactated Ringer's solution, and

5) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min.

The following will then be performed in the chamber:

1) subject enters the chamber for an hour, and

2) during the exposure, the subject rates symptoms.

Fifteen min after exposure, the following will be performed:

1) subject rates symptoms,

2) clinical examination of the nose, eyes, and throat, and rating of signs,

3) sample of nasal secretions taken, as before exposure, and

4) sample of tear fluid taken, as before exposure.

This procedure will be repeated during the four days of exposure in a series. On the day after the fourth exposure of a series, the subject will fill out an RQLQ and return it by mail or phone in the answers.

Data Analysis: Ratings of symptoms (scores per site and total score) will be analyzed for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis.

Score on the ROLQ will be analyzed for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis.

Ratings from the clinical exam will be analyzed similarly as the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions will be assessed for the markers albumin, IL-8, and sICAM. The quantities will be compared for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

As noted at the outset, this study concerns statistical power. From a practical standpoint with respect to Phase 3b, these analyses will boil down to assessment of the number of subjects needed to establish meaningful effects in the study of exposure to chloropicrin.

Phase 3b - Daily Exposures, Signs and Symptoms of Irritation, and Evidence of Inflammation: The work in Phase 3b will build upon that of Phase 3a, but will entail exposure of subjects to chloropicrin.

Objective: To establish in 16 males and 16 females, screened for normal mucosal condition, whether mildly irritating ambient exposures to chloropicrin of one hour per day over four days lead to: a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The levels of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways. (The numbers 16 males and 16 females may require modification since results of Phase 3a will determine the number of subjects needed for criterion levels of power.)

Apparatus: Chloropicrin (CCl_3NO_2; CAS #76-06-2) will be the test material. Exposures will take place in the chambers. Concentration will be controlled as in Phase 2. Other apparatus will include that used in screening.

Exposures will entail two concentrations of chloropicrin and a blank. The lower concentration of chloropicrin will equal 0.1 ppm (100 ppb), the occupational threshold limit value (TLV). The higher concentration will equal 0.15 ppm (150 ppb).

Procedure: Subjects will serve in three blocks of six sessions, each beginning on a Friday and ending on a Friday. Exposures to a given concentration in the chambers will last one hour on four successive days, Monday through Thursday. In one block, a subject will have exposure to chloropicrin at 0.1 ppm. In another block, the subject will have exposure to 0.15 ppm, and, in a third block, the subject will have exposure to just air on the four days. The order in which a given subject has exposure to the three conditions

will vary to prevent confounding of order of exposure with level. Except for personnel who set and monitor the conditions in the chamber, the personnel who will deal with the subjects and the subjects themselves will be blinded to the conditions at any given time. At least one week will separate the end of one block and the beginning of another for a subject.

On the first Friday of a block, baseline measures will be taken (see Table 3). The following will be performed:

1) a Rhinoprobe sample taken from the inferior turbinate in one nostril to establish the number and kind of cells in the mucosal layer (see details under **7. Human Subjects**),

2) subject fills out the RQLQ,

3) subject rates symptoms (see Table 1),

4) clinical examination of the nose, eyes, and throat, and rating of signs,

5) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, followed by nasal lavage, and

6) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min,

On Monday through Thursday, the following will be performed before the subject enters the chamber:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination of the nose, eyes, and throat, and rating of signs,

4) sample of nasal secretions taken,

5) sample of tear fluid will be taken,

6) exhaled nitric oxide (NO) measured via the mouth while exhaling through an expiratory resistance to indicate NO generated in the lungs,

7) office spirometry to establish forced vital capacity (FVC) and forced expiratory volume at 1 sec (FEV_1), and

8) nasal resistance measured by active, anterior rhinomanometry.

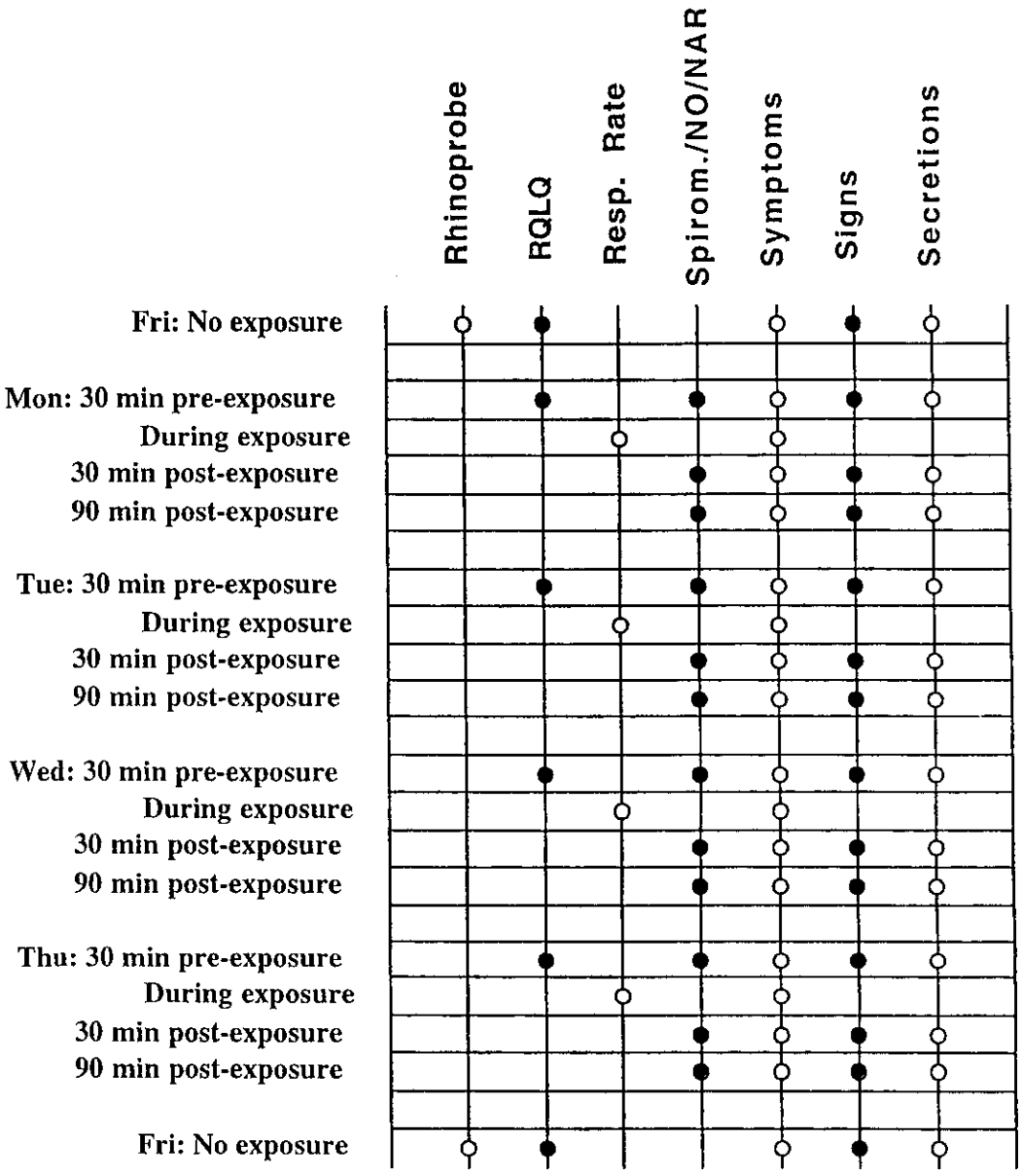
The following will then be performed in the chamber:

1) subject enters the chamber,

2) respiratory rate monitored remotely via Respibands placed around the thorax, and

3) in the last half-minute of exposure, the subject rates symptoms.

Table 3


Rhinoprobe
RQLQ
Resp. Rate
Spirom./NO/NAR
Symptoms
Signs
Secretions
Fri: No exposure
Mon: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Tue: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Wed: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Thu: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Fri: No exposure

Thirty min and 90 min after a session, the following will be performed:

1) subject rates symptoms,

2) clinical examination, with rating of signs,

3) nasal secretions taken, as before the session ,

4) tear fluid taken, as before the session,

4) exhaled NO measured, as before the session

5) office spirometry, as before the session, and

7) measurement of nasal resistance, as before the session.

On Tuesday, Wednesday, and Thursday, the cycle will be repeated, i.e., a pre-session evaluation and two post-session evaluations.

Approximately 24 hr after the Thursday session, the subject will return and the following performed:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination, with ratings of signs,

4) nasal secretions collected, and

5) Rhinoprobe sample taken from the opposite nostril from the first.

Data Analysis: For interpretation of the study, we will distinguish between variables meant to monitor safety and those of substantive interest.

With respect to safety:

Respiratory rate will be monitored to examine whether the subject remains relaxed during exposures. An unexpected rise can indicate anxiety and gives reason to query the subject about any perceived threat.

Results of the office spirometry will be compared before and after exposure to examine whether a subject has experienced bronchoconstriction from the exposure. A reduction of FEV_1 of 15 % will be reason to remove a subject from further exposures at the level that caused the constriction.

Exhaled nitric oxide (NO) will be compared between after and before to examine whether a subject has developed any inflammation in the lungs.

With respect to substantive interest:

Score on the RQLQ will be analyzed for effects of level of exposure (0 ppm, 0.1 ppm, 0.15 ppm), of day as an indicator of total exposure, and of sex.

Ratings of symptoms (scores per site and total score) will be analyzed for effects of level of exposure, of time since exposure (30 min and 90 min post-exposure), of day as an indicator of total exposure, and of sex.

Ratings of signs on the clinical examination will be analyzed similarly to the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions from both the nose and the eye will be assessed for total mass collected and for the markers albumin, IL-8, and sICAM. The quantities of the biochemical indices will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

Nasal resistance will be compared before vs. after exposure as an aspect of dose of chloropicrin vapor. A blocked nasal passage means lower dose than a patent passage. An increase in resistance is a normal response to inhalation of an irritating vapor. Nasal resistance will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex.

The cells in the Rhinoprobe samples will be compared from the first to the sixth days as an index of cumulative effect of exposure.

7. Human Subjects

Inclusion Criteria for Normal Subjects:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

4) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Normal Subjects:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses.

8) Abuse of alcohol (more than three drinks of alcohol a day over the last year).

9) Use of mood altering drugs within the last year.

10) Use of tobacco or smoking of any substance including marijuana within the at year.

11) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

12) Impaired sense of smell.

13) For Phase 3b, pregnancy at the time of the study.

14) Evidence of active infection, rhinitis, pharyngitis, clinically-significant inflammation in the nose or throat, or certain structural abnormalities in these regions.

15) Evidence of conjunctivitis, abnormal redness of the eyes, or abnormalities of the surface of the eyes.

16) Clinically elevated nasal resistance.

17) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of any of these conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

Sense of Smell: Screening for smell will entail taking a standardized test, e.g., the Connecticut Chemosensory Clinical Research Center [CCCRC] Test for odor threshold and odor identification (Cain, 1989). Subjects will excluded if their olfactory ability falls below normal by the criteria of the test.

Nasal Resistance: The assessment of nasal resistance will occur both in screening and in testing in Phase 3b. Nasal resistance will be measured via a system of computerized anterior rhinomanometry (RHINO; MultiSpiro, San Clemente, CA) that avoids deformation of the nares. The system relies upon an oxygen-type face-mask to monitor flow from one nostril while a tube sealed via a pressure patch (Rhino Diagnostics, Inc., San Diego, CA) monitors pressure at the other nostril.

During testing, the subject breathes normally through the mask through four cycles per nostril. Signals for pressure and flow are digitized and used to calculate resistance at –1.5 cm water column. Subjects with clinically abnormal resistance, defined as >5 cm H_20/ L/sec will be excluded in screening.

Nasal Cytology: Nasal cytology will be used both in screening and in testing in Phase 3b. Subjects who pass the clinical screening examination of the nose, throat, and eyes may still show evidence of inflammation in cytological analysis. In order to obtain cells, the investigator will use a Rhinoprobe, a flexible curette with a 1-mm diam. cup. Sampling with the Rhinoprobe entails a gentle scrape of 3-mm length along the superficial nasal mucosa of the lower turbinate under visual inspection. The procedure causes minor discomfort for an instant.

For analysis, the specimen is gently spread over a small area of a microscope slide, fixed in 95% alcohol, and stained with Wright-Giemsa stain. It is examined under low power (100 x) to determine the adequacy of the specimen and the areas of interest and then graded under high power (1000 x) for cells.

Nasal cytology can reveal various conditions, as follows (see Table 4): 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection, 3) large numbers of eosinophils or basophilic cells (3+ or 4+) provide evidence of inflammation. When used as an outcome variable in testing in Phase 3b, cells will be counted exactly.

Spirometry: Spirometry will be used both in screening and in testing in Phase 3b. Office spirometry testing will conform to the recommendations of the American Thoracic Society. Data to be acquired from this test include the following: FVC, the total volume of gas exhaled after a full inspiration, and FEV_1, the gas volume exhaled in one second by a forced expiration from a full inspiration. The data will allow calculation of the ratio FEV_1/FVC. Subjects whose pulmonary function fails to lie at or above 75% of predicted FEV_1 or FVC will be excluded.

Ocular Cytology: Ocular cytology will be performed in screening on impression-samples taken from the conjunctival membrane inside the lower eyelid. The lower lid

will be sampled with a 3-mm diam. membrane filter placed at the end of a rod. The rod weighs 60 g and suspension of the rod within a short outer cylinder at the moment of contact insures that 60 g will be exerted on the lid. The samples will be analyzed for the presence of cells in the same way as in nasal cytology.

Table 4
Guide for Grading Nasal Cytograms

Quantitative Analysis	Semi-quantitative Analysis	Grade
Epithelial Cells		
N/A	Normal morphology	N
N/A	Abnormal morphology	A
N/A	Ciliocytophthoria	CCP
Eosinophils, neutrophils		
0*	None	0
0.1-1.0*	Occasional cells	0.5+
1.1-5.0*	Few scattered cells or small clumps	1+
5.1-15.0*	Moderate number of cells and larger clumps	2+
15.1-20.0*	Larger clumps of cells which do not cover the entire field	3+
>20*	Large clumps of cells covering the entire field	4+
Basophilic cells		
0*	None	0
0.1-0.3*	Occasional cells	0.5+
0.4-1.0*	Few scattered cells	1+
1.1-3.0*	Moderate number of cells	2+
3.1-6.0*	Many cells easily seen	3+
>6.0*	Large number of cells, as many as 25 per high power field	4+
Bacteria**		
N/A	None seen	0
N/A	Occasional clump	1+
N/A	Moderate number	2+
N/A	Many easily seen	3+
N/A	Large numbers covering the entire field	4+
Goblet cells***		
0	None	0
1-24%	Occasional to few cells	1+
25-49%	Moderate number	2+
50-74%	Many easily seen	3+
75-100%	Large number, may cover entire field	4+

* Mean of cells per 10 high power fields (x1000)
** Note presence of intracellular bacteria
*** Ratio of goblet cells to epithelial cells, expressed as percentage

Persons with significantly elevated levels of PMNs (neutrophils, eosinophils, and basophilic cells) in the conjunctival membrane will be excluded. Persons with small elevations above normal will not.

Collection of Nasal Secretions: Nasal secretions will be collected in two ways, via placement of a 6-mm diam. sponge on the septum for 30 sec and via nasal lavage. Nasal lavage will follow the collection of undiluted secretions by the sponge. The lavage entails instillation of 5 ml of sterile lactated ringer's solution into each nostril using a pipette. Participants are instructed to tilt their heads backward approximately 30 degrees from horizontal while in the seated position. As the lactated ringer's solution is instilled participants are instructed to hold their breath and refrain from swallowing. After 10 seconds the participant is asked to expel the resulting mixture of mucus and lactated ringer's into a collection vessel. The nasal lavage samples will be used to look for evidence of inflammatory mediators and to compare their levels with those found on the samples collected via sponge.

Pregnancy: In Phase 3b, testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded. (Pregnant females can participate in Phase 3a.)

Inclusion Criteria for Subjects with Allergic Rhinitis:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must show evidence of allergic rhinitis.

4) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

5) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Subjects with Allergic Rhinitis:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses.

7) History of ocular abnormalities, other than a need for glasses.

8) Abuse of alcohol (more than three drinks of alcohol a day over the last year).

9) Use of mood altering drugs within the last year.

10) Use of tobacco or smoking of any substance including marijuana within the at year.

11) Daily use of medication, excluding birth control pills.

12) Absent sense of smell.

13) Evidence of active infection or certain structural abnormalities in the upper airways.

14) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of signs of allergic rhinitis should be present in the patients. Signs of other conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

As part of the examination, the examiner will also establish if there is a history of seasonal or perennial allergic rhinitis with positive skin test to one or more allergens within previous 12 mo. If skin test results are unavailable, a test will be performed at the Nasal Dysfunction Clinic under the supervision of Dr. Terence Davidson. Positive evidence will be a criterion for inclusion. The examiner will also ask about symptoms. A score ≥ 8 for the combined nasal and eye symptoms, with congestion score ≥ 2 for at least three of the five days prior to screening will be a criterion for inclusion.

Sense of Smell: Screening for smell will entail the same testing as described for normals. Subjects will excluded if their olfactory ability falls into the anosmic zone, i.e., if the subject evinces no olfactory ability whatsoever.

Nasal Resistance: The assessment of nasal resistance will entail the same testing as described above. Subjects will be excluded if their resistance lies above 6 H_2O/L/sec.

Nasal Cytology: Nasal cytology will be performed as described above. Subjects will be excluded if they show the following in their cytograms: 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for

respiratory viral infection, or 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection.

Spirometry: Screening will entail the same methods as described for normals.

Ocular Cytology: Screening will be performed by the methods described above for normals. Subjects will not be excluded by this test, except for signs of infection, but the results will be compared with those of the nasal cytology for possible stratification of the sample of subjects by presence or absence of inflammatory cells in the eye.

8. Informed Consent

Subjects will be recruited from the general population. Formal consent will be sought just prior to screening, typically by staff involved in the screening tests. The consent form will contain the relevant facts regarding the reason for the study, the task at hand, any possible adverse effects, and the opportunity to withdraw at any time without penalty. Consent will be written and the subject will receive a copy of the form along with the subject's bill of rights.

9. Therapeutic Alternatives

Not a therapeutic study.

10. Potential Risks

Screening: The screening procedures of direct visualization of the nasal and ocular mucosae and measurement of nasal resistance pose no realistic risks that we can anticipate. Sampling from the nasal mucosa has been performed routinely by Dr. Jalowayski in the Nasal Dysfunction Clinic for many years and entails minimal risk. The impression cytological sampling from the lower lid, also entails minimal risk.

Testing: The risks associated with testing entail sensory irritation of the upper airways or eyes and remotely in these circumstances asthma. We say "remotely" because the exposures will be to perithreshold concentrations with respect to irritation.

11. Risk Management

Monitoring: Subjects will be monitored visually during testing. Testing will stop if the subject finds the test situation uncomfortable and asks to stop. A staff member will guide the subject away from any exposure in progress. We will instruct subjects to report any respiratory symptoms immediately.

Actions to be taken if an adverse event occurs:

Upper Airway or Ocular Discomfort: Irritation of the nose or eyes should subside soon after exposure. Should effects last longer than expected, the subject will be instructed regarding how to irrigate the affected area with saline solution. An eye irrigation system exists within the laboratory. Furthermore, over-the-counter

preparations for such purposes will be available on-site for the subject to use before leaving the lab and to take home. The subject will be informed that if any such irritation or discomfort fails to show progressive improvement during the ensuing hours, then the subject should page Dr. Terence Davidson who will oversee medical management of the symptoms.

Lower airway reactions: Should exposure cause shortness of breath from apparent bronchoconstriction, then an ACLS-certified staff member will initiate an asthma-management protocol. An emergency kit to treat acute asthma will include: a) a Ventolin inhaler (albuterol USP inhalation aerosol), b) an AeroChamber to be used with the metered dose inhaler, c) a nebulizer and oxygen source, d) a pulse oxymeter, and e) an epinephrine auto-injector (EpiPen) to deliver a 0.3 mg intramuscular dose of epinephrine (1:1000), and d) apparatus for oxygen therapy.

The protocol for management of acute asthma will be the following: a) if symptoms occur, the subject will be offered treatment with Ventolin (one puff held in the lungs for 10 sec., followed by a second puff 30 sec later), b) the pulse oxymeter will be attached to a digit in order to monitor oxygen saturation, c) if the subject seems unable to inhale from the inhaler adequately, then the nebulizer will be used, d) Dr. Thomas Bruff will be paged and informed of the event, and he will decide how the subject should be managed, e) if the subject is not in acute respiratory distress, he will be asked to remain in the lab for the next hour; the subject will be given Dr. Bruff's beeper number when released;; Dr. Bruff will contact the patient within 2-4 hours and will arrange any further care, including office follow-up, if necessary f) if the subject fails to obtain relief, he will be transported to the emergency room at Thornton Hospital, less than 2 miles from the lab; management of the subject in the ER will depend upon their standard protocols; and g) if the subject shows signs of anaphylactic shock while at the laboratory, he can be given an injection of epinephrine from the EpiPen and administered humidified oxygen; in that case, 911 will be called.

12. Potential Benefits

There will be no benefits to individual subjects.

This research is motivated by the need to develop a definitive set of data relevant to environmental and occupational exposures to chloropicrin. Since the chemical has very low systemic toxicity compared to its rather strong irritating properties, it is irritation that will dictate permissible exposure levels. (The irritation response, as we have noted, largely protects against systemic exposure.) In so far as the present research makes it possible to set more scientifically defensible exposures, then its potential benefit to occupational and public health is considerable.

Approximately 50% of the ACGIH TLV's are based on sensory irritation and yet there have been almost no controlled studies of such. Companies and trade groups that exercise product stewardship over commodity chemicals seek quantitative data on human sensory irritation. As product stewards, these entities are often asked to advise users of their products about hazardous properties. Indeed, they must do so in Material Safety

Data Sheets. This matter goes beyond setting permissible exposure levels into more intrinsic understanding of the perceptual and physiological effects of the chemicals. We see these developments as salutary.

13. Risk-Benefit Ratio: Minor risk, no individual benefits, but benefits to society as indicated just above.

14. Expense to subject: None anticipated.

15. Payment for Participation: Subjects will receive $15 per hour for participation. subjects can receive their payment (cash) at the end of a session or can allow it to accumulate over sessions by mutual agreement. Subjects will not be reimbursed for travel.

16. Privileges/Certifications and Licenses: Dr. Davidson has privileges at UCSD Medical Center. He indicated to the Human Subjects Program in 1999 that sampling from mucosal tissue, the only procedure where we touch the subject, does not require direct medical supervision. Dr. Jalowayski, who has worked with Dr. Davidson in the Nasal Dysfunction Clinic for more than 15 years, developed the sampling and performs it routinely in the clinic. Dr. Jalowayski will do the sampling in this project.

Dr. Roland Schmidt is a Ph.D. biologist with no privileges.

Dr. Thomas Bruff, board certified in internal medicine and in occupational medicine, is attending staff at UCSD Medical Center. He is ACLS-certified.

17. Bibliography:

American Conference of Governmental Industrial Hygienists (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th ed. Cincinnati: ACGIH.

Cain, W. S. (1989). Testing olfaction in a clinical setting. Ear, Nose, and Throat Journal, 68, 316-328.

Calderon, M. A, Devalia, J. L, Prior, A. J, Sapsford, R. J, & Davies, R. (1997). A comparison of cytokine release from epithelial cells cultured from biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis..Journal of Allergy and Clinical Immunology, 99, 65-76.

Ciprandi, G., Buscaglia, S., Pesce, G., Villaggio, B., Bagnasco, M., & Canonica, G.W. (1993). Allergic subjects express intercellular adhesion molecule – 1(ICAM-1 or CD54 on epithelial cells of conjunctiva after allergen challenge. Journal of Allergy and Clinical Immunology, 91(2), 783-792.

Ciprandi, G, Pronzato, C, Passalacqua, G, Ricca, V, Bagnasco, M, Grogen, J, Mela, G. S. & Canonica, G. W. (1996). Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells: An antiallergic

activity. Journal of Allergy Clinical Immunology, 98(6), 1088-96.

Granstrand, P., Nylander-French, L., & Holmstreom, M. (1998). Biomarkers of nasal inflammation in wood-surface coating industry worker. American Journal of Industrial Medicine, 33(4), 392-9.

Jalowayski, A. A. & Zeiger, R.S. (1988). Examination of nasal or conjunctival epithelium specimens. In Manual of Allergy and Immunology, eds. Lawlor, G. J. and Fischer, T.J.; Little, Brown and Co. Boston/Toronto. Pp. 432-434.

Juniper, E. F., Thompson, A. K., Ferrie, P. J., & Roberts, J. N. (2000). Development and validation of the Mini Rhinoconjunctivitis Quality of Life Questionnaire. Clinical and Experimental Allergy, 30, 132-140.

Klimek, L, Wolf, H, Mewes, T, Dormann, D, Reske-Kunz, A, Schnitker, J & Mann, W. (1999). The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophilic cationic protein and tryptase in nasal secretions. Journal of Allergy and Clinical Immunology, 103, 47-53.

Kobayashi, R. H., Beaucher, W. N., Koepke, J. W., Luskin, A. et al. (1995). Triamcinolone acetonide aqueous nasal spray for the treatment of patients with perennial allergic rhinitis: A multicenter, randomized, double-blind, pacebo-controlled study. Clinical Therapeutics, 17, 503-513.

Krieger, R. I. (1996). An assessment of implied worker exposure and risk associated with chloropicrin loading, application, and field tarping activities following application, and implied exposure and risk for off-field concentrations resulting from soil fumigation. Registration document submitted to CAL-EPA Department of Pesticide Regulation, October 1996.

Meltzer. E.O., Jalowayski, A.A., Orgel, H.A., & Harris, A. G. (1998). Subjective and objective assessments in patients with seasonal allergic rhinitis: Effects of therapy with mometasone furoate nasal spray. Journal of Allergy and Clinical Immunology, 102 (1), 39-49.

Polito, A. J. & Proud, D. (1998). Epithelial cells as regulators of airway inflammation. Journal of Allergy and Clinical Immunology, 102(5), 714-718.

Reichelmann, H., Deutschle, T., Friemel, E., Gross, M.-J., & Bachem, M. (2003). Biological markers in nasal secretions. Eur. Respir. J., 21, 600-605.

Silkoff, P. E., Robbins, R. A., Gaston, B., Lundberg, J. O. N., & Townley, R. G. (2000). Endogenous nitric oxide in allergic airway disease. Journal of Allergy and Clinical Immunology, 105 (3), 438-448.

U. S. Environmental Protection Agency (1984). Notice to Manufacturers, Formulators, Producers and Registrants of Fumigant Products. PR Notice-84-5.

Van Cauwenberge, P., Juniper, E. F., & the STAR Investigating Group (2000) Comparison of the efficacy, safety and quality of life provided by fexofenadine hydrochloride 120 mg, laratadine 10 mg and placebo administered once daily for the treatment of seasonal allergic rhinitis. Clinical and Experimental Allergy, 30, 891-899.

Wagenmann, M., Baroody, F. M., Desrosiers, M., Hubbard, W. S., Ford, S., Lichtenstein, L. M., & Naclerio, R. M. (1996). Unilateral nasal allergen challenge leads to bilateral release of prostaglandin D_2. Clinical and Experimental Allergy, 26, 371-378.

Wilson, S. J, Lau, L., & Howarth, P. H. (1998). Inflammatory mediators in naturally occurring rhinitis. Clinical and Experimental Allergy, 28(2), 220-7.

18. Industry Studies: Supported by the Chloropicrin Manufacturer's Task Force. The Task Force approached the Chemosensory Perception Laboratory about the need for the information and we designed the study. The UCSD Office of Contracts and Grants Administration negotiated a contract with the Task Force. The University will retain all patent rights to the results and is free to publish the work. The PI has no personal agreements (e.g., consultation, confidentiality) with the Chloropicrin Manufacturer's Task Force.

19. Other Funding: None.

20. Cancer Studies: Not applicable.

21. Biological Materials Transfer Agreement: No materials to be transported.

22. Investigational Drug Fact Sheet: No investigational drugs to be used.

23. Conflict of Interest: Neither the PI nor any of the investigators has a conflict of interest regarding the sponsor, CMTF.

24. Nursing Staff: No impact.

25. Information Service: Will be completed.

030246
PENDING

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN RESEARCH PROTECTIONS PROGRAM

Date: August 1, 2003

To: Dr. William Cain Mailcode: 0957

Re: Project #030246
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

Your July 15, 2003 response to the Committee's letter of March 7, 2003 has been received and reviewed. However, there are still some issues that require clarification.

1. 700-U forms Statement of Economic Interest have been submitted for Dr. Cain, Dr. Jalowayski and Dr. Schmidt, but this office has not received any potential conflict of interest information for Drs. Davidson and Bruff. As currently written, the research plan, under the Conflict of Interest section, simply states, "Forms submitted here." The Conflict of Interest Office at UCSD has indicated that they have not yet received paperwork for this project. You are requested to revise the research plan at this section to discuss whether or not any research team member has a financial relationship with the sponsoring company. In addition, when available, you are requested to forward a copy of the COI concurrence letter received from the COI office.

Please send your reply to the attention of: Human Research Protections Program Office, mail code 0052. Final approval will be forwarded just as soon as we can determine that your responses are satisfactory.

Sincerely,

D. Masys

/nm

Daniel Masys, M.D., Director
Human Research Protections Program
Phone: 858-455-5050
E-mail: hrpp@ucsd.edu

030246

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

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Reply to:
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9500 GILMAN DRIVE
LA JOLLA, CALIFORNIA 92093

Date: 15 July 2003

To: Dr. Daniel Masys, Human Research Protections Program

JUL 17 2003

Re: Protocol #030246, Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Masys:

1. Regarding Phases 1 and 2, I indicated on the notification that I returned that 126 persons had participated and that there had been no injuries or unexpected reactions that involved risk to human subjects. I did, however, make reference to one subject who thought exposure to chloropicrin might have made a cold worse. I documented in detail the contacts with this subject until resolution of the issue.

   In the revised application, I state explicitly that there were no adverse events in Phases I and II, that subjects tolerated the exposures, and that no subjects terminated because of an inability to tolerate chloropicrin. This information was implicit by my focus on the substantive aspects of the study.

2. We have made a decision to eliminate WS3 from the study for practical reasons. Instead we plan to use vapor from a Sunbeam Health at Home Waterless Vaporizer. We will use the device as indicated by the manufacturer. The vapor emitted from the vaporizer will come from heat-activated scent pads that contain essential oils with menthol, camphor, and eucalyptus. These are the materials used in many over-the-counter preparations to relieve stuffiness, e.g., Vicks. Although the exact composition of the scent pads is a trade secret, we have every reason to believe that exposure to the vapor will pose no known risks to health. I have contacted Sunbeam and they have written back that the materials are natural and safe to the best of their knowledge.

   We have modified the application and consent form to reflect this change in plan and have revised the consent form to conform to wording in the "policy on the Use of Women of Child-Bearing Potential in Drug Studies." Because we see no risk to women of child-bearing potential in exposure to the blend, we have eliminated testing for pregnancy in phase 3a, but have retained it in phase 3b, where subjects will be exposed to chloropicrin.

3. We have made reference to Dr. Roland Schmidt in the relevant section

4. The 730-U forms have been included.

5. The application and consent form now indicate how a subject may withdraw from an exposure.

6. The consent form now states that subjects will be informed of any significant research findings relevant to their continued participation.

7. The additional minor revisions to the consent have been made.

Yours truly,

William S. Cain

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Title of Project: Human Sensory Irritation Testing for Chloropicrin

Principal Investigator: William S. Cain, Ph.D., Professor of Surgery (Otolaryngology)

Co-Investigators: Alfredo Jalowayski, Ph.D., Research Specialist, Depts. of Pediatrics and Surgery (Otolaryngology), Terence M. Davidson, M.D., Professor of Surgery (Otolaryngology-HNS), Thomas Bruff, M.D., M.P.H., Assistant Clinical Professor of Medicine, and Roland Schmidt, Ph.D., Project Scientist, Dept. of Surgery (Otolaryngology)

1. Facilities: The work will be performed in the Chemosensory Perception Laboratory at the La Jolla Village Professional Center.

2. Duration: One to two years.

3. Specific Aims: There are three phases of work, each with a specific aim, as follows:

Aim 1: To establish the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the sites. **(Completed.)**

Aim 2: To establish sensitivity for ambient exposures where all channels for detection are available and, within this framework, to establish whether sensitivity varies among exposures of different durations, ranging from a few seconds to 30 minutes. Of particular interest with respect to time-course is whether undetectable concentrations become detectable over time. **(Completed.)**

Aim 3: To establish whether mildly irritating ambient exposures of one-hour per day over four days lead to a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. **(This aim is the subject of the current IRB application.)**

Aims 1 and 2 pertained to all persons, those exposed adventitiously from environmental releases of chloropicrin and those exposed occupationally. Aim 3 pertains primarily to persons exposed occupationally and more likely to have repetitive exposure. Aim 3 can also apply to residential exposure because off-gassing may occur for several days from a single field. If there are multiple fields in an area, residents could conceivably be exposed intermittently for a longer time.

4. Background and Significance

Chloropicrin is a colorless liquid with a sharp, penetrating odor. The material is used primarily to fumigate fields in order to control soil-borne fungi, plant diseases, and nematodes. Pre-planting soil fumigation with chloropicrin makes it possible for plants such as strawberries to achieve exceptional root-growth unaffected by soil-pests and disease. Because of the sharpness of its vapors, chloropicrin has also been added to

other, odorless fumigants, such as sulfuryl flouride and methyl bromide as a warning agent. The same property of sharpness has led to use of the material as an agent to test the vapor attenuating properties of activated carbon and the fit of personal protective respiratory devices.

The mammalian acute and chronic toxicology of chloropicrin, including that following inhalation, is well described and current. Testing in laboratory animals by inhalation or other routes of exposure has indicated that chloropicrin in nonsystemically toxic exposures poses no hazard to pregnancy or to the developing fetus, nor does it impair reproductive functioning. It has been found to be noncarcinogenic following lifetime inhalation exposure to rats and mice and in chronic feeding studies to beagle dogs. A chronic oral (gavage) study in rats produced a single animal with a stomach papilloma in the test group receiving 10mg/kg/day, the highest dose tested. This papilloma is considered to be spontaneous in origin. There is no evidence that chloropicrin will bioaccumulate in mammalian cells.

Human beings come into contact with chloropicrin principally on the job in agriculture or, to a lesser extent, in wood preservation. The American Conference of Governmental Industrial Hygienists (ACGIH) initially set the threshold limit value (TLV) at 1 ppm (time-weighted average, TWA) in 1957 and reduced it to 0.1 ppm in 1959, where it remains today. In the documentation, ACGIH states: "A TLV-TWA of 0.1 ppm is recommended for repeated exposure to chloropicrin to prevent eye irritation and the potential for pulmonary changes" (p. 299). OSHA in the U.S. and its regulatory counterparts in many industrial countries also use a TWA of 0.1 ppm as the permissible exposure limit.

The repellent properties of chloropicrin at low levels include reflex blepharospasm, tearing, and pungency that can effectively function as warning properties. As Krieger (1996) noted in an exposure and risk assessment: "This protective reflex response is an important homeostatic mechanism by which exposure is reduced and the sensitive human pulmonary system is spared adverse effects resulting from higher levels of chloropicrin. ...It is a protective reflex response that occurs when chloropicrin contacts the trigeminal nerves of the moist nasal airways. Tearing (lachrymation) and painful stinging eyes results from temporary disturbances of the eye that are completely reversible and occur at concentrations of 0.15 ppm to 0.3 ppm chloropicrin. This undeniable reflex response warns persons to move to uncontaminated environments before toxic exposures occur." (Pp. 10-11.)

Krieger noted further that the mandated use of chloropicrin to warn of the presence of odorless fumigants "gives clear evidence of regulatory recognition of the importance and usefulness of the warning properties of low levels of chloropicrin" (p. 12). To illustrate, U. S. EPA PR Notice 84-5 describes the language to be used for methyl bromide that contains chloropicrin: "This product contains chloropicrin as a warning odorant. Chloropicrin may be irritating to the upper respiratory tract, and even at low levels can cause painful irritation to the eyes, producing tearing. If these symptoms occur, leave the fumigation area immediately." (U. S. EPA, 1984; p. A-1).

The table below, from Krieger (1996), shows human responses to airborne chloropicrin at various levels and at exposures from instantaneous to chronic to the extent that these have been studied. In the main, the effects derive from direct contact between chloropicrin and tissue at the portal of entry. The nausea and vomiting that may occur from high levels of exposure are thought to occur because of swallowing of the irritating material that has dissolved into saliva.

Until the present investigation, human responses to airborne chloropicrin have been known mainly through anecdotal reports or from studies and other observations collected many decades ago. These experiences indicated that exposure to airborne chloropicrin concentrations of about 0.15 ppm to 0.3 ppm can cause tearing and eye irritation that is reversible upon cessation of exposure.

The results of these studies will provide an appropriate framework to learn whether odor or sensory irritation that signals low-level exposure to chloropicrin provides an adequate mechanism to prevent occurrence of adverse effects seen in high-dose animal studies. This work also will allow evaluation of important human attributes of the responses to low-level chloropicrin; individual variability in responses; differences in sensitivity as a function of modality of response (ocular stimulation vs, nasal stimulation); and repeated vs. single exposure responses that may be important manifestations of the sensory response to chloropicrin.

Together, the information from these studies will be useful not only in human hazard assessment, but also for the promulgation of exposure standards for workers and the general population.

This research plan was devised in response to a request for proposals from the Chloropicrin Manufacturers Task Force. The protocol was, however, created by the investigators at UCSD.

Human Responses to Airborne Chloropicrin Exposures[1]

Exposures	Conc. x Time	Response	Characteristics
Workplace or Off-Site	Less than TLV-TWA 0.1 ppm or less	None No Adverse effects (eye): NOAEL	None
Instantaneous Contact	0.15 to 0.3 ppm x secs	*Common* Chemical *Sense Threshold*	Tolerable *with very slight or no irritating sensation*
		Reflex tearing and reflex coughing	① Concentration dependent
			②Response ceases when exposure removed
			③Response non-cumulative
			④Primary irritant, little or no systemic effect
	TLV-TWA: 0.1 ppm or less		
Acute	Greater than TLV-TWA of 0.1 ppm; seconds-minutes	Irritation	*Slightly irritating, some stinging or burning of eye, nose, throat*
	0.15-0.3 ppm; 3.30 secs.	Lacrimation	
	0.3-0.37 ppm; 2-2.5 seconds	Eye irritation; LOAEL	
	0.9 ppm	Odor threshold	
	1.5 ppm; few seconds		⑤ Secondary airway irritant; lacrimation, nausea, vomiting
	4 ppm; few seconds	Pulmonary Toxicity	Upper respiratory tract irritation; tissue injury; edema
Chronic	0.1 ppm	No Effect	None

[1]To the best of our knowledge, no controlled human studies of chloropicrin exposure have been completed. Each of these values in this table is anecdotal or derives from a source for which analytical verification of chloropicrin concentrations and standardized evaluation of subject response does dot exist. The present protocol describes a laboratory study that incorporates proper and comprehensive control of variables as well as appropriate analytical and psychophysical response measurement technology to assure valid results having the greatest degree of scientific certainty..

5. Progress

We have collected the data regarding aims 1 (Phase 1) and 2 (Phase 2), but have not yet finished the analysis. The total number of participants who passed screening equaled 126, half males and half females. Almost all of these persons participated in exposures in Phase 1, Phase 2, or both. A small number of people did not go on to either phase, an occurrence we see in every study that entails some time between screening and further participation.

No adverse events were reported in Phases 1 or 2. Subjects tolerated the exposures and no subject terminated because of an inability to tolerate chloropicrin. We believe this reflects our focus on just-detectable stimulation.

As we did note in a file submitted with the application for renewal, one subject who developed a cold before his one day of exposure in Phase 1 speculated that his exposure to chloropicrin may have exacerbated the cold. He raised this issue because his cold was more severe than that of his roommate from whom he thought he caught the cold. The cold resolved in two weeks. This occurred at the end of the school year and the student left the area, so the question of whether he and we might have agreed on his further participation never came up. Since the protocol had specified that we would not run subjects who had "acute illness in the previous month" and since this subject with the beginning symptoms of the cold had sneaked through, not at screening, but at the day of testing, we realized the need to be more vigilant. We need to note, however, that we had no reason to believe that the testing would harm a person with a common cold. Since that event, any subject who indicates more than minimal symptoms before testing has a further interview with a doctoral level professional who goes into details and decides whether the subject may continue. Since that time we have postponed testing three persons until symptoms possibly indicative of a cold have resolved. Testing has then proceeded normally.

Phase 1 focused upon determination of the threshold for odor, for feel in the eyes, and feel in the nose (see Figure 1 for the subject's task of choosing which of three cones at a station had odor). The threshold for odor equaled 750 ppb, averaged over the 63 participants in this phase. The threshold for feel in the eyes equaled 929 ppb (30-sec exposure). And, the threshold for feel in the nose proved indeterminate over the range of concentrations explored. It was therefore clear from the testing that for the average subject, the eyes were more sensitive. The threshold for odor proved to be a little lower than previous estimates and the threshold for feel in the eyes proved to be somewhat higher. Nevertheless, as Phase 2 indicated, the threshold for feel in the eyes is quite time-dependent. We obtained enough data from each subject to make meaningful comparisons of the individual differences that underlie the average threshold. This will be an important part of our analysis over the next months.

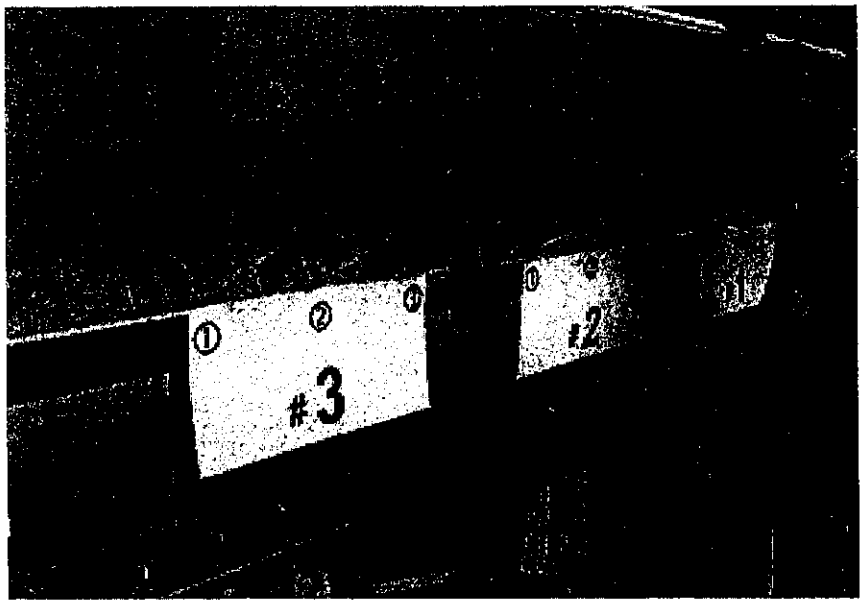


Figure 1. Showing a subject interacting with the delivery device used to present vapors for short exposures (2 sec for odor and nasal feel; 30 sec for feel in the eyes). A computer controlled voice guides the subject through the task.

In Phase 2, a total of 62 subjects sought to detect the presence of feel from chloropicrin in the environment of a chamber and to rate confidence in their judgments of detect-no detect (see Fig. 2). In some exposures chloropicrin was present and in some it was not. (The levels were too low for the subjects to detect any odor.) In this phase we found that over time subjects could detect feel in the eyes at much lower concentrations than the 929 ppb felt in Phase 1. Subjects could, for example, eventually feel a concentration of 75 ppb after many minutes of exposure. Demonstration of the time-dependence of detection of irritation is important new information regarding chloropicrin. As in the testing in Phase 1, individual differences in sensitivity will be a focus of analysis.

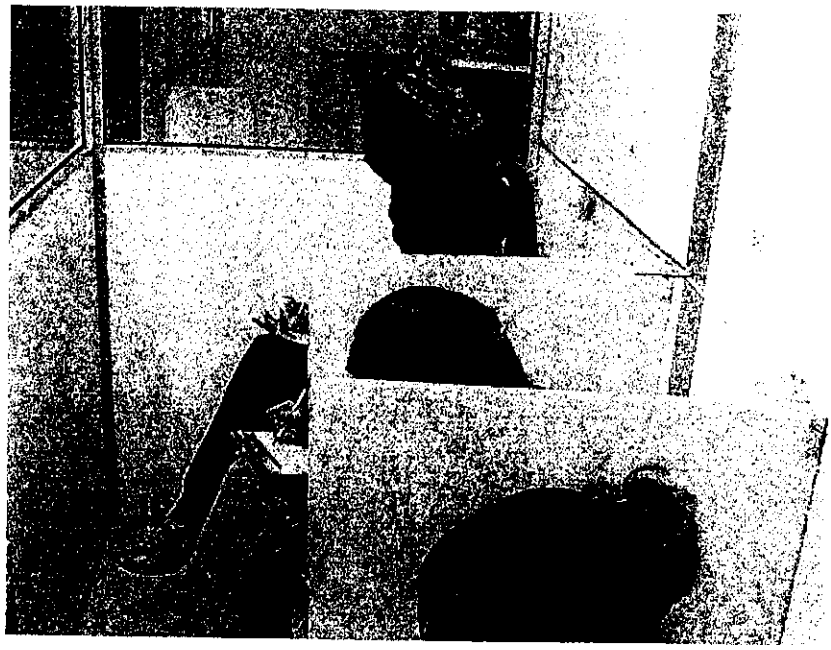


Figure 2. Subjects in the setting for Phase 2, where they sought to detect presence of chloropicrin over time. They made judgments every minute for 20-30 minute sessions. This setting will also be used for the experiments of Phase 3.

As we analyze the results of Phases 1 and 2, we are prepared to move into Phase 3.

Phase 3: Signs/Symptoms of Irritation in Daily Exposures

Objective: To establish whether mildly irritating ambient exposures of one hour per day over four days lead to: a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The level of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways.

The objective will be addressed in two stages, Phase 3a and Phase 3b.

Phase 3a - Preliminary Study to Validate Techniques of Measurement: This phase will serve to establish: a) sensitivity and stability of clinical assessment of signs and symptoms, b) sensitivity and stability of three assays for biochemical markers, and c) sensitivity and stability of the Rhinconjunctivitis Quality of Life Questionnaire in the context of the present work (e.g., Juniper, Thompson, Ferrie, & Roberts, 2000). Statistically speaking, the preliminary study deals with issues of the power to detect effects of exposure to chloropicrin when these exist, but the preliminary study of Phase 3a will entail no exposure to chloropicrin. It will instead set the stage for exposures to chloropicrin in Phase 3b.

Clinical Studies of Rhinitis: In some respects, the agenda for Phase 3 resembles that of clinical studies of effects of medication on allergic rhinitis. In view of the

uniqueness of the present work, the similarities to and differences from those clinical studies seem worthy of comment. In such studies, investigators may challenge subjects with allergens in order to induce reactions and may measure how medication diminishes sensitivity or reactivity. Outcome variables of interest generally include a score for signs/symptoms and perhaps an index of quality of life, such as the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), and some objective indices, such as biochemical markers of inflammation. Variables of this nature have relevance to the present research. In the present case, however, a major question concerns whether subjects will become symptomatic and show signs of rhinitis from environmentally plausible exposures rather than whether they will become less symptomatic from medication. If they become symptomatic, then the outcome variables need to show the result and its various manifestations with clarity. If they do not, then the investigators need to show that the outcome variables would have registered a positive result if it had occurred. This accounts for concerns over sensitivity and stability.

Positive Control Group vs. Positive Control Exposure: Clinical studies provoke symptoms and signs in allergic subjects under conditions well characterized with respect to agent and duration of effect. Hence, when an investigator sprays a dilute solution of ragweed pollen extract into the nose of a person with known allergy to that substance, he can anticipate that an ensuing acute episode may begin within minutes and subside after an hour. The protocol may even entail increasing the concentration of the challenging agent until a response occurs. This establishes the positive control. The study of a noxious vapor cannot easily follow this simple route. It is unclear how to provoke rhinitis in a normal person. An irritating vapor may do so, but at this time we know neither the vapor nor the level that will do so unfailingly. Nor can we know how to provoke the symptoms as temporarily as one might in persons with allergic rhinitis. How then can one demonstrate the sensitivity to resolve the presence vs. the absence of rhinitis in the study of a noxious vapor? One way would be to demonstrate the ability of the outcome measurements to resolve between normals and persons already symptomatic. For this, one can study persons screened for normal nasal health and persons screened for presence of symptomatic allergic rhinitis. By this device, a positive control group substitutes for a positive control exposure.

Ratings: In clinical studies of rhinitis, investigators rather commonly ask subjects to fill out simple ratings of symptoms such as that shown in Table 1 below. The scale reportedly picks up differences of approximately half a step in nasal score with power greater than 90% in groups of a dozen or so subjects, but the published literature contains little documentation of such sensitivity (Meltzer, personal communication).

Although indispensable in clinical studies, ratings of symptoms fail to capture the effects of symptoms on everyday life. This situation has given rise to questionnaires that assess quality of life. The RQLQ, a self-administered questionnaire of high reliability (Cronbach's alpha >0.90) and validity, assesses quality of life as pertains to the nose and eyes. Via a series of 28 questions it assesses how "nose/eye symptoms trouble you in your life." Regarding sleep, for instance, it inquires: "How troubled have you been by each of these sleep problems during the last week as a result of your nose/eye symptoms? 4. Difficulty getting to sleep. [The respondent has six choices per item ranging from *not*

troubled to *extremely troubled*] 5. Wake up during night. 6. Lack of a good night's sleep." Most commonly, ratings of quality of life ask the respondent to consider the previous six or seven days. In general, the RQLQ is not given on sequential days, but we see no a prioi reason why it would not work well even though many days of the frame of reference, a week, would overlap.

Clinical studies of rhinitis include ratings from clinicians as well as ratings from subjects. Although the ratings of the clinicians include signs, such as congestion of the nose and tearing of the eyes, they also include the impressions of the subjects. Consequently, it hardly surprises that the ratings of the subjects and the ratings of the clinicians agree extremely well (see Meltzer et al., 1998). In a study that cannot blind the subject from the nature of an exposure, it seems essential to blind the clinician. The clinician can use a rating scale not unlike that of the subject, but should remain oblivious to conditions of exposure to remain unbiased.

Biochemical Markers: Irrespective of whether it arises from allergies, infection, or irritation, rhinitis represents an inflammation of the nasal mucosa. The presence of inflammation reveals itself via the type and number of cells that can be sampled from the mucosa. In samples taken by Rhinoprobe and quantified as number of cells per high power field (HPF), normal superficial nasal mucosa has the following cytologic profile: neutrophils 0-10.5, eosinophils 0-0.45, and basophilic cells 0-0.2 (Jalowayski & Zeiger, 1988). When neutrophils reach 16-20, eosinophils reach 1.1-5.0, and basophilic cells reach 0.4-1.0, subjects characteristically exhibit medically significant inflammation.

The presence of inflammation in mucosal tissue can also reveal itself via levels of biochemical markers. Those shown to increase significantly in nasal and conjunctival mucosa, nasal secretions, and tear fluid after challenge with allergens include albumin, interleukin-8 (IL-8) and soluble intercellular adhesion molecule (sICAM) (Ciprandi, Pronzato, Passalacqua, Ricca, et al., 1996; Calderon, Devalia, Prior, Sapsford, & Davies, 1997; Granstrand, Nylander-French, & Holmstreom, 1998; Wilson, Lau, & Howarth, 1998). Albumin leaks across the mucosal layer during an inflammatory process. Its concentration in nasal fluid and tears collected via absorbent sponge can be measured by ELISA. IL-8, one of several proinflammatory cytokines that play a major role in attracting and activating inflammatory cells, can also be measured by ELISA. sICAM, known to play a role in eosinophil and neutrophil infiltration across endothelial and epithelial cells, can also be measured by ELISA.

The accuracy of research on markers in secretions can depend upon the mode of collection, a constantly evolving matter as investigators abandon error-prone methods. The present research will entail collection of fluid with small cellulose sponges. Such collection does not irritate and avoids uncertainties regarding dilution as in the method of nasal lavage. Weight of the sponge before and after 30-sec application to the mucosa gives an exact measure of amount of fluid collected. The sponge also elutes albumin better than filter paper, another medium of collection. Because collection via the sponge is new, few data address sensitivity to analytes except for eosinophilic cationic protein (ECP) and tryptase in secretions (Klimek, Wolf, Mewes, Dormann, et al., 1999; Riechelmann, Deutschle, Friemel, et al., 2003). We believe that we will collect sufficient

quantities of nasal and tear fluid to measure levels of albumin, IL-8, and sICAM accurately. The preliminary study will test ability to resolve between normals and symptomatic persons. In order to assess the sensitivity of our assay for samples taken via sponge, however, we must also include use of a nasal lavage. Its use will lie under the rubric of secretions, as indicated in Table 3.

Preview of the Preliminary Study: The study described in its particulars below will entail the following: Two groups of subjects, one screened for absence of nasal inflammation and the other for presence of allergic rhinitis will participate in six sessions of testing on contiguous workdays, with each session approximately 2.5 hours long. On days two through five, the subjects will have one-hour exposures to vapor from the scent pads (essential oils of menthol, camphor, and eucalyptus) of a Sunbeam Health at Home Vaporizer. The product literature describes the vapor as "a blend of soothing essential oils." Furthermore, we have been in touch with the consumer division of the Sunbeam Corp. and they have stated that the ingredients are natural, and nontoxic to the best of their knowledge. Subjects should experience no irritation. Subjects will have a clinical exam of the nose, eyes, and throat before each exposure and twice after the exposure, within a half-hour and after another hour. At the approximate times when subjects will have the clinical exams, they will also rate their symptoms. In connection with the clinical exams, the examiner will collect fluid from the nose and eyes for analysis of the markers albumin, IL-8, and sICAM. Before an exposure and 24 hours after the fourth exposure in a block, the subject will fill out the RQLQ with respect to the previous 24 hours.

Questions addressed in the preliminary study will include:

1) How well will a blinded clinical exam resolve between the normals and the subjects with rhinitis? We expect that the exam will resolve between the groups, but the variability day to day, across groups, and across exposures should yield important statistical information about the sensitivity and stability of the exam.

2) Will the RQLQ perform meaningfully for resolution between normals and persons with rhinitis when given repeatedly?

3) Will secretions collected with sponges provide enough material for reliable and sensitive assays for the markers albumin, IL-8, and sICAM?

Stated as an objective, Phase 3a entails the following:

Objective: To establish in 32 subjects (16 males and 16 females), half of them (eight males and eight females) screened for normal mucosal condition and the other half for allergic rhinitis, the stability and sensitivity of various tests to reflect the presence of mucosal inflammation.

Table 1

Rating of Symptoms

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Collected by:_____

Use the following scale to indicate the degree of symptoms:

Scale	Degree	Meaning
0	None	No symptom.
1	Mild	Symptom present, but minimal awareness, easily tolerated.
2	Moderate	Symptom definite and bothersome, but tolerated.
3	Severe	Symptom hard to tolerate and can interfere with activities of daily living or sleeping.

Nose

a. Congestion ____
b. Runny Nose ____
c. Itchiness/Sneezing ____
d. Irritation ____ ____

Eye

a. Tearing ____
b. Puffiness ____
c. Itchiness ____
d. Irritation ____ ____

Throat

a. Cough ____
b. Hoarseness ____
c. Dryness ____
d. Irritation ____ ____

Total Score ____

Table 2

Rating of Signs

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Examined by:_____

Use the following scale to indicate the degree of clinical signs:

Scale	Degree
0	No sign evident.
1	Sign barely present.
2	Sign clearly present.
3	Sign quite marked.

Nose
a. Congestion ____
b. Rhinorrhea ____
c. Erythema ____ ____

Eye
a. Tearing ____
b. Puffiness ____
c. Erythema ____ ____

Throat
a. Cough ____
b. Swelling ____
c. Erythema ____ ____

Total Score ____

Apparatus: Exposures will take place in the chambers, with the Sunbeam Waterless Vaporizer operating during the exposures. The vaporizer causes no change in ambient temperature or humidity. Its small heater merely promotes evaporation from a 1" x 2" scent pad. Other apparatus will include that used in screening (see details under **7. Human Subjects**).

Procedure: Screening will establish two groups of subjects, 16 with and 16 without evidence of nasal inflammation (see details under **7. Human Subjects**). Every subject will participate in the same tests that will revolve around four exposures of one-hour in the chambers.

On each day of exposure, the following will be performed before exposure begins (details of exams appears under **7. Human Subjects**):

1) subject fills out the RQLQ,

2) subject rates symptoms (see Table 1),

3) clinical examination of the nose, eyes, and throat, and rating of signs (see Table 2),

4) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, followed by nasal lavage with 10 ml of lactated Ringer's solution, and

5) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min.

The following will then be performed in the chamber:

1) subject enters the chamber for an hour, and

2) during the exposure, the subject rates symptoms.

Fifteen min after exposure, the following will be performed:

1) subject rates symptoms,

2) clinical examination of the nose, eyes, and throat, and rating of signs,

3) sample of nasal secretions taken, as before exposure, and

4) sample of tear fluid taken, as before exposure.

This procedure will be repeated during the four days of exposure in a series. On the day after the fourth exposure of a series, the subject will fill out an RQLQ and return it by mail or phone in the answers.

Data Analysis: Ratings of symptoms (scores per site and total score) will be analyzed for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis.

Score on the ROLQ will be analyzed for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis.

Ratings from the clinical exam will be analyzed similarly as the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions will be assessed for the markers albumin, IL-8, and sICAM. The quantities will be compared for reliability and for ability to distinguish persons screened for normality vs. those screened for allergic rhinitis. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

As noted at the outset, this study concerns statistical power. From a practical standpoint with respect to Phase 3b, these analyses will boil down to assessment of the number of subjects needed to establish meaningful effects in the study of exposure to chloropicrin.

Phase 3b - Daily Exposures, Signs and Symptoms of Irritation, and Evidence of Inflammation: The work in Phase 3b will build upon that of Phase 3a, but will entail exposure of subjects to chloropicrin.

Objective: To establish in 16 males and 16 females, screened for normal mucosal condition, whether mildly irritating ambient exposures to chloropicrin of one hour per day over four days lead to: a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The levels of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways. (The numbers 16 males and 16 females may require modification since results of Phase 3a will determine the number of subjects needed for criterion levels of power.)

Apparatus: Chloropicrin (CCl_3NO_2; CAS #76-06-2) will be the test material. Exposures will take place in the chambers. Concentration will be controlled as in Phase 2. Other apparatus will include that used in screening.

Exposures will entail two concentrations of chloropicrin and a blank. The lower concentration of chloropicrin will equal 0.1 ppm (100 ppb), the occupational threshold limit value (TLV). The higher concentration will equal 0.15 ppm (150 ppb).

Procedure: Subjects will serve in three blocks of six sessions, each beginning on a Friday and ending on a Friday. Exposures to a given concentration in the chambers will last one hour on four successive days, Monday through Thursday. In one block, a subject will have exposure to chloropicrin at 0.1 ppm. In another block, the subject will have exposure to 0.15 ppm, and, in a third block, the subject will have exposure to just air on the four days. The order in which a given subject has exposure to the three conditions

will vary to prevent confounding of order of exposure with level. Except for personnel who set and monitor the conditions in the chamber, the personnel who will deal with the subjects and the subjects themselves will be blinded to the conditions at any given time. At least one week will separate the end of one block and the beginning of another for a subject.

On the first Friday of a block, baseline measures will be taken (see Table 3). The following will be performed:

1) a Rhinoprobe sample taken from the inferior turbinate in one nostril to establish the number and kind of cells in the mucosal layer (see details under **7. Human Subjects**),

2) subject fills out the RQLQ,

3) subject rates symptoms (see Table 1),

4) clinical examination of the nose, eyes, and throat, and rating of signs,

5) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, followed by nasal lavage, and

6) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min,

On Monday through Thursday, the following will be performed before the subject enters the chamber:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination of the nose, eyes, and throat, and rating of signs,

4) sample of nasal secretions taken,

5) sample of tear fluid will be taken,

6) exhaled nitric oxide (NO) measured via the mouth while exhaling through an expiratory resistance to indicate NO generated in the lungs,

7) office spirometry to establish forced vital capacity (FVC) and forced expiratory volume at 1 sec (FEV_1), and

8) nasal resistance measured by active, anterior rhinomanometry.

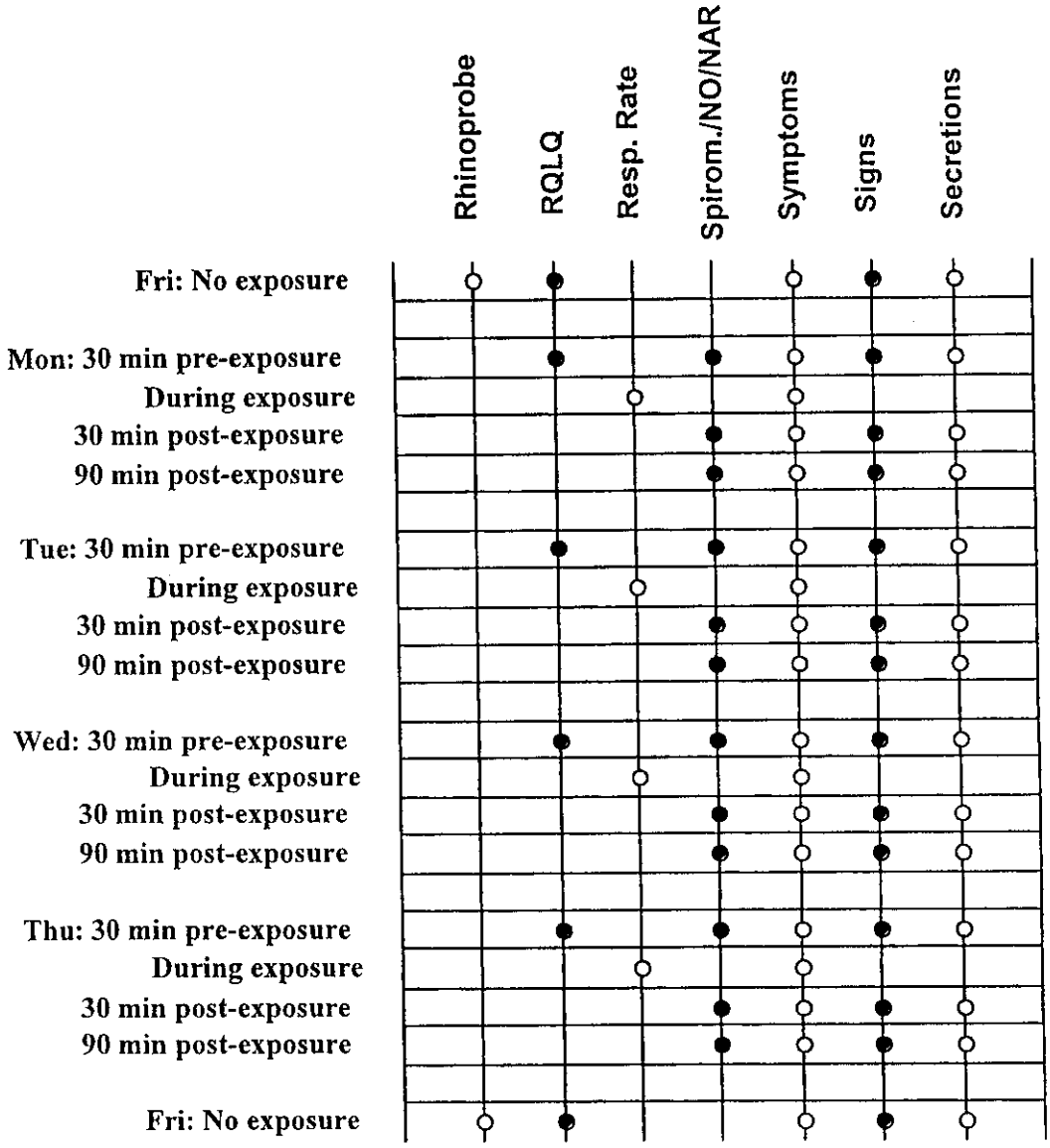
The following will then be performed in the chamber:

1) subject enters the chamber,

2) respiratory rate monitored remotely via Respibands placed around the thorax, and

3) in the last half-minute of exposure, the subject rates symptoms.

Table 3


Rhinoprobe
RQLQ
Resp. Rate
Spirom./NO/NAR
Symptoms
Signs
Secretions
Fri: No exposure
Mon: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Tue: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Wed: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Thu: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Fri: No exposure

Thirty min and 90 min after a session, the following will be performed:

1) subject rates symptoms,

2) clinical examination, with rating of signs,

3) nasal secretions taken, as before the session ,

4) tear fluid taken, as before the session,

1) exhaled NO measured, as before the session

2) office spirometry, as before the session, and

7) measurement of nasal resistance, as before the session.

On Tuesday, Wednesday, and Thursday, the cycle will be repeated, i.e., a pre-session evaluation and two post-session evaluations.

Approximately 24 hr after the Thursday session, the subject will return and the following performed:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination, with ratings of signs,

4) nasal secretions collected, and

5) Rhinoprobe sample taken from the opposite nostril from the first.

Data Analysis: For interpretation of the study, we will distinguish between variables meant to monitor safety and those of substantive interest.

With respect to safety:

Respiratory rate will be monitored to examine whether the subject remains relaxed during exposures. An unexpected rise can indicate anxiety and gives reason to query the subject about any perceived threat.

Results of the office spirometry will be compared before and after exposure to examine whether a subject has experienced bronchoconstriction from the exposure. A reduction of FEV_1 of 15 % will be reason to remove a subject from further exposures at the level that caused the constriction.

Exhaled nitric oxide (NO) will be compared between after and before to examine whether a subject has developed any inflammation in the lungs.

With respect to substantive interest:

Score on the RQLQ will be analyzed for effects of level of exposure (0 ppm, 0.1 ppm, 0.15 ppm), of day as an indicator of total exposure, and of sex.

Ratings of symptoms (scores per site and total score) will be analyzed for effects of level of exposure, of time since exposure (30 min and 90 min post-exposure), of day as an indicator of total exposure, and of sex.

Ratings of signs on the clinical examination will be analyzed similarly to the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions from both the nose and the eye will be assessed for total mass collected and for the markers albumin, IL-8, and sICAM. The quantities of the biochemical indices will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

Nasal resistance will be compared before vs. after exposure as an aspect of dose of chloropicrin vapor. A blocked nasal passage means lower dose than a patent passage. An increase in resistance is a normal response to inhalation of an irritating vapor. Nasal resistance will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex.

The cells in the Rhinoprobe samples will be compared from the first to the sixth days as an index of cumulative effect of exposure.

7. Human Subjects

Inclusion Criteria for Normal Subjects:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

4) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Normal Subjects:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses.

8) Abuse of alcohol (more than three drinks of alcohol a day over the last year).

9) Use of mood altering drugs within the last year.

10) Use of tobacco or smoking of any substance including marijuana within the at year.

11) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

12) Impaired sense of smell.

13) For Phase 3b, pregnancy at the time of the study.

14) Evidence of active infection, rhinitis, pharyngitis, clinically-significant inflammation in the nose or throat, or certain structural abnormalities in these regions.

15) Evidence of conjunctivitis, abnormal redness of the eyes, or abnormalities of the surface of the eyes.

16) Clinically elevated nasal resistance.

17) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of any of these conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

Sense of Smell: Screening for smell will entail taking a standardized test, e.g., the Connecticut Chemosensory Clinical Research Center [CCCRC] Test for odor threshold and odor identification (Cain, 1989). Subjects will excluded if their olfactory ability falls below normal by the criteria of the test.

Nasal Resistance: The assessment of nasal resistance will occur both in screening and in testing in Phase 3b. Nasal resistance will be measured via a system of computerized anterior rhinomanometry (RHINO; MultiSpiro, San Clemente, CA) that avoids deformation of the nares. The system relies upon an oxygen-type face-mask to monitor flow from one nostril while a tube sealed via a pressure patch (Rhino Diagnostics, Inc., San Diego, CA) monitors pressure at the other nostril.

During testing, the subject breathes normally through the mask through four cycles per nostril. Signals for pressure and flow are digitized and used to calculate resistance at –1.5 cm water column. Subjects with clinically abnormal resistance, defined as >5 cm H_20/ L/sec will be excluded in screening.

Nasal Cytology: Nasal cytology will be used both in screening and in testing in Phase 3b. Subjects who pass the clinical screening examination of the nose, throat, and eyes may still show evidence of inflammation in cytological analysis. In order to obtain cells, the investigator will use a Rhinoprobe, a flexible curette with a 1-mm diam. cup. Sampling with the Rhinoprobe entails a gentle scrape of 3-mm length along the superficial nasal mucosa of the lower turbinate under visual inspection. The procedure causes minor discomfort for an instant.

For analysis, the specimen is gently spread over a small area of a microscope slide, fixed in 95% alcohol, and stained with Wright-Giemsa stain. It is examined under low power (100 x) to determine the adequacy of the specimen and the areas of interest and then graded under high power (1000 x) for cells.

Nasal cytology can reveal various conditions, as follows (see Table 4): 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection, 3) large numbers of eosinophils or basophilic cells (3+ or 4+) provide evidence of inflammation. When used as an outcome variable in testing in Phase 3b, cells will be counted exactly.

Spirometry: Spirometry will be used both in screening and in testing in Phase 3b. Office spirometry testing will conform to the recommendations of the American Thoracic Society. Data to be acquired from this test include the following: FVC, the total volume of gas exhaled after a full inspiration, and FEV_1, the gas volume exhaled in one second by a forced expiration from a full inspiration. The data will allow calculation of the ratio FEV_1/FVC. Subjects whose pulmonary function fails to lie at or above 75% of predicted FEV_1 or FVC will be excluded.

Ocular Cytology: Ocular cytology will be performed in screening on impression-samples taken from the conjunctival membrane inside the lower eyelid. The lower lid

will be sampled with a 3-mm diam. membrane filter placed at the end of a rod. The rod weighs 60 g and suspension of the rod within a short outer cylinder at the moment of contact insures that 60 g will be exerted on the lid. The samples will be analyzed for the presence of cells in the same way as in nasal cytology.

Table 4
Guide for Grading Nasal Cytograms

Quantitative Analysis	Semi-quantitative Analysis	Grade
Epithelial Cells		
N/A	Normal morphology	N
N/A	Abnormal morphology	A
N/A	Ciliocytophthoria	CCP
Eosinophils, neutrophils		
0*	None	0
0.1-1.0*	Occasional cells	0.5+
1.1-5.0*	Few scattered cells or small clumps	1+
5.1-15.0*	Moderate number of cells and larger clumps	2+
15.1-20.0*	Larger clumps of cells which do not cover the entire field	3+
>20*	Large clumps of cells covering the entire field	4+
Basophilic cells		
0*	None	0
0.1-0.3*	Occasional cells	0.5+
0.4-1.0*	Few scattered cells	1+
1.1-3.0*	Moderate number of cells	2+
3.1-6.0*	Many cells easily seen	3+
>6.0*	Large number of cells, as many as 25 per high power field	4+
Bacteria**		
N/A	None seen	0
N/A	Occasional clump	1+
N/A	Moderate number	2+
N/A	Many easily seen	3+
N/A	Large numbers covering the entire field	4+
Goblet cells ***		
0	None	0
1-24%	Occasional to few cells	1+
25-49%	Moderate number	2+
50-74%	Many easily seen	3+
75-100%	Large number, may cover entire field	4+

* Mean of cells per 10 high power fields (x1000)
** Note presence of intracellular bacteria
*** Ratio of goblet cells to epithelial cells, expressed as percentage

Persons with significantly elevated levels of PMNs (neutrophils, eosinophils, and basophilic cells) in the conjunctival membrane will be excluded. Persons with small elevations above normal will not.

Collection of Nasal Secretions: Nasal secretions will be collected in two ways, via placement of a 6-mm diam. sponge on the septum for 30 sec and via nasal lavage. Nasal lavage will follow the collection of undiluted secretions by the sponge. The lavage entails instillation of 5 ml of sterile lactated ringer's solution into each nostril using a pipette. Participants are instructed to tilt their heads backward approximately 30 degrees from horizontal while in the seated position. As the lactated ringer's solution is instilled participants are instructed to hold their breath and refrain from swallowing. After 10 seconds the participant is asked to expel the resulting mixture of mucus and lactated ringer's into a collection vessel. The nasal lavage samples will be used to look for evidence of inflammatory mediators and to compare their levels with those found on the samples collected via sponge.

Pregnancy: In Phase 3b, testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded. (Pregnant females can participate in Phase 3a.)

Inclusion Criteria for Subjects with Allergic Rhinitis:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must show evidence of allergic rhinitis.

4) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

5) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Subjects with Allergic Rhinitis:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses.

7) History of ocular abnormalities, other than a need for glasses.

8) Abuse of alcohol (more than three drinks of alcohol a day over the last year).

9) Use of mood altering drugs within the last year.

10) Use of tobacco or smoking of any substance including marijuana within the at year.

11) Daily use of medication, excluding birth control pills.

12) Absent sense of smell.

13) Evidence of active infection or certain structural abnormalities in the upper airways.

14) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of signs of allergic rhinitis should be present in the patients. Signs of other conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

As part of the examination, the examiner will also establish if there is a history of seasonal or perennial allergic rhinitis with positive skin test to one or more allergens within previous 12 mo. If skin test results are unavailable, a test will be performed at the Nasal Dysfunction Clinic under the supervision of Dr. Terence Davidson. Positive evidence will be a criterion for inclusion. The examiner will also ask about symptoms. A score >8 for the combined nasal and eye symptoms, with congestion score >2 for at least three of the five days prior to screening will be a criterion for inclusion.

Sense of Smell: Screening for smell will entail the same testing as described for normals. Subjects will excluded if their olfactory ability falls into the anosmic zone, i.e., if the subject evinces no olfactory ability whatsoever.

Nasal Resistance: The assessment of nasal resistance will entail the same testing as described above. Subjects will be excluded if their resistance lies above 6 H_2O/L/sec.

Nasal Cytology: Nasal cytology will be performed as described above. Subjects will be excluded if they show the following in their cytograms: 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for

respiratory viral infection, or 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection.

Spirometry: Screening will entail the same methods as described for normals.

Ocular Cytology: Screening will be performed by the methods described above for normals. Subjects will not be excluded by this test, except for signs of infection, but the results will be compared with those of the nasal cytology for possible stratification of the sample of subjects by presence or absence of inflammatory cells in the eye.

8. Informed Consent

Subjects will be recruited from the general population. Formal consent will be sought just prior to screening, typically by staff involved in the screening tests. The consent form will contain the relevant facts regarding the reason for the study, the task at hand, any possible adverse effects, and the opportunity to withdraw at any time without penalty. Consent will be written and the subject will receive a copy of the form along with the subject's bill of rights.

9. Therapeutic Alternatives

Not a therapeutic study.

10. Potential Risks

Screening: The screening procedures of direct visualization of the nasal and ocular mucosae and measurement of nasal resistance pose no realistic risks that we can anticipate. Sampling from the nasal mucosa has been performed routinely by Dr. Jalowayski in the Nasal Dysfunction Clinic for many years and entails minimal risk. The impression cytological sampling from the lower lid, also entails minimal risk.

Testing: The risks associated with testing entail sensory irritation of the upper airways or eyes and remotely in these circumstances asthma. We say "remotely" because the exposures will be to perithreshold concentrations with respect to irritation.

11. Risk Management

Monitoring: Subjects will be monitored visually during testing. Testing will stop if the subject finds the test situation uncomfortable and asks to stop. A staff member will guide the subject away from any exposure in progress. We will instruct subjects to report any respiratory symptoms immediately.

Actions to be taken if an adverse event occurs:

Upper Airway or Ocular Discomfort: Irritation of the nose or eyes should subside soon after exposure. Should effects last longer than expected, the subject will be

instructed regarding how to irrigate the affected area with saline solution. An eye irrigation system exists within the laboratory. Furthermore, over-the-counter preparations for such purposes will be available on-site for the subject to use before leaving the lab and to take home. The subject will be informed that if any such irritation or discomfort fails to show progressive improvement during the ensuing hours, then the subject should page Dr. Terence Davidson who will oversee medical management of the symptoms.

Lower airway reactions: Should exposure cause shortness of breath from apparent bronchoconstriction, then an ACLS-certified staff member will initiate an asthma-management protocol. An emergency kit to treat acute asthma will include: a) a Ventolin inhaler (albuterol USP inhalation aerosol), b) an AeroChamber to be used with the metered dose inhaler, c) a nebulizer and oxygen source, d) a pulse oxymeter, and e) an epinephrine auto-injector (EpiPen) to deliver a 0.3 mg intramuscular dose of epinephrine (1:1000), and d) apparatus for oxygen therapy.

The protocol for management of acute asthma will be the following: a) if symptoms occur, the subject will be offered treatment with Ventolin (one puff held in the lungs for 10 sec., followed by a second puff 30 sec later), b) the pulse oxymeter will be attached to a digit in order to monitor oxygen saturation, c) if the subject seems unable to inhale from the inhaler adequately, then the nebulizer will be used, d) Dr. Thomas Bruff will be paged and informed of the event, and he will decide how the subject should be managed, e) if the subject is not in acute respiratory distress, he will be asked to remain in the lab for the next hour; the subject will be given Dr. Bruff's beeper number when released;; Dr. Bruff will contact the patient within 2-4 hours and will arrange any further care, including office follow-up, if necessary f) if the subject fails to obtain relief, he will be transported to the emergency room at Thornton Hospital, less than 2 miles from the lab; management of the subject in the ER will depend upon their standard protocols; and g) if the subject shows signs of anaphylactic shock while at the laboratory, he can be given an injection of epinephrine from the EpiPen and administered humidified oxygen; in that case, 911 will be called.

12. Potential Benefits

There will be no benefits to individual subjects.

This research is motivated by the need to develop a definitive set of data relevant to environmental and occupational exposures to chloropicrin. Since the chemical has very low systemic toxicity compared to its rather strong irritating properties, it is irritation that will dictate permissible exposure levels. (The irritation response, as we have noted, largely protects against systemic exposure.) In so far as the present research makes it possible to set more scientifically defensible exposures, then its potential benefit to occupational and public health is considerable.

Approximately 50% of the ACGIH TLV's are based on sensory irritation and yet there have been almost no controlled studies of such. Companies and trade groups that exercise product stewardship over commodity chemicals seek quantitative data on human

sensory irritation. As product stewards, these entities are often asked to advise users of their products about hazardous properties. Indeed, they must do so in Material Safety Data Sheets. This matter goes beyond setting permissible exposure levels into more intrinsic understanding of the perceptual and physiological effects of the chemicals. We see these developments as salutary.

13. Risk-Benefit Ratio: Minor risk, no individual benefits, but benefits to society as indicated just above.

14. Expense to subject: None anticipated.

15. Payment for Participation: Subjects will receive $15 per hour for participation. subjects can receive their payment (cash) at the end of a session or can allow it to accumulate over sessions by mutual agreement. Subjects will not be reimbursed for travel.

16. Privileges/Certifications and Licenses: Dr. Davidson has privileges at UCSD Medical Center. He indicated to the Human Subjects Program in 1999 that sampling from mucosal tissue, the only procedure where we touch the subject, does not require direct medical supervision. Dr. Jalowayski, who has worked with Dr. Davidson in the Nasal Dysfunction Clinic for more than 15 years, developed the sampling and performs it routinely in the clinic. Dr. Jalowayski will do the sampling in this project.

Dr. Roland Schmidt is a Ph.D. biologist with no privileges.

Dr. Thomas Bruff, board certified in internal medicine and in occupational medicine, is attending staff at UCSD Medical Center. He is ACLS-certified.

17. Bibliography:

American Conference of Governmental Industrial Hygienists (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th ed. Cincinnati: ACGIH.

Cain, W. S. (1989). Testing olfaction in a clinical setting. Ear, Nose, and Throat Journal, 68, 316-328.

Calderon, M. A, Devalia, J. L, Prior, A. J, Sapsford, R. J, & Davies, R. (1997). A comparison of cytokine release from epithelial cells cultured from biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis..Journal of Allergy and Clinical Immunology, 99, 65-76.

Ciprandi, G., Buscaglia, S., Pesce, G., Villaggio, B., Bagnasco, M., & Canonica, G.W. (1993). Allergic subjects express intercellular adhesion molecule – 1(ICAM-1 or CD54 on epithelial cells of conjunctiva after allergen challenge. Journal of Allergy and Clinical Immunology, 91(2), 783-792.

Ciprandi, G, Pronzato, C, Passalacqua, G, Ricca, V, Bagnasco, M, Grogen, J,

Mela, G. S. & Canonica, G. W. (1996). Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells: An antiallergic activity. Journal of Allergy Clinical Immunology, 98(6), 1088-96.

Granstrand, P., Nylander-French, L., & Holmstreom, M. (1998). Biomarkers of nasal inflammation in wood-surface coating industry worker. American Journal of Industrial Medicine, 33(4), 392-9.

Jalowayski, A. A. & Zeiger, R.S. (1988). Examination of nasal or conjunctival epithelium specimens. In Manual of Allergy and Immunology, eds. Lawlor, G. J. and Fischer, T.J.; Little, Brown and Co. Boston/Toronto. Pp. 432-434.

Juniper, E. F., Thompson, A. K., Ferrie, P. J., & Roberts, J. N. (2000). Development and validation of the Mini Rhinoconjunctivitis Quality of Life Questionnaire. Clinical and Experimental Allergy, 30, 132-140.

Klimek, L, Wolf, H, Mewes, T, Dormann, D, Reske-Kunz, A, Schnitker, J & Mann, W. (1999). The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophilic cationic protein and tryptase in nasal secretions. Journal of Allergy and Clinical Immunology, 103, 47-53.

Kobayashi, R. H., Beaucher, W. N., Koepke, J. W., Luskin, A. et al. (1995). Triamcinolone acetonide aqueous nasal spray for the treatment of patients with perennial allergic rhinitis: A multicenter, randomized, double-blind, pacebo-controlled study. Clinical Therapeutics, 17, 503-513.

Krieger, R. I. (1996). An assessment of implied worker exposure and risk associated with chloropicrin loading, application, and field tarping activities following application, and implied exposure and risk for off-field concentrations resulting from soil fumigation. Registration document submitted to CAL-EPA Department of Pesticide Regulation, October 1996.

Meltzer. E.O., Jalowayski, A.A., Orgel, H.A., & Harris, A. G. (1998). Subjective and objective assessments in patients with seasonal allergic rhinitis: Effects of therapy with mometasone furoate nasal spray. Journal of Allergy and Clinical Immunology, 102 (1), 39-49.

Polito, A. J. & Proud, D. (1998). Epithelial cells as regulators of airway inflammation. Journal of Allergy and Clinical Immunology, 102(5), 714-718.

Reichelmann, H., Deutschle, T., Friemel, E., Gross, M.-J., & Bachem, M. (2003). Biological markers in nasal secretions. Eur. Respir. J., 21, 600-605.

Silkoff, P. E., Robbins, R. A., Gaston, B., Lundberg, J. O. N., & Townley, R. G. (2000). Endogenous nitric oxide in allergic airway disease. Journal of Allergy and Clinical Immunology, 105 (3), 438-448.

U. S. Environmental Protection Agency (1984). Notice to Manufacturers, Formulators, Producers and Registrants of Fumigant Products. PR Notice-84-5.

Van Cauwenberge, P., Juniper, E. F., & the STAR Investigating Group (2000) Comparison of the efficacy, safety and quality of life provided by fexofenadine hydrochloride 120 mg, laratadine 10 mg and placebo administered once daily for the treatment of seasonal allergic rhinitis. Clinical and Experimental Allergy, 30, 891-899.

Wagenmann, M., Baroody, F. M., Desrosiers, M., Hubbard, W. S., Ford, S., Lichtenstein, L. M., & Naclerio, R. M. (1996). Unilateral nasal allergen challenge leads to bilateral release of prostaglandin D_2. Clinical and Experimental Allergy, 26, 371-378.

Wilson, S. J, Lau, L., & Howarth, P. H. (1998). Inflammatory mediators in naturally occurring rhinitis. Clinical and Experimental Allergy, 28(2), 220-7.

18. Industry Studies: Supported by the Chloropicrin Manufacturer's Task Force. The Task Force approached the Chemosensory Perception Laboratory about the need for the information and we designed the study. The UCSD Office of Contracts and Grants Administration negotiated a contract with the Task Force. The University will retain all patent rights to the results and is free to publish the work. The PI has no personal agreements (e.g., consultation, confidentiality) with the Chloropicrin Manufacturer's Task Force.

19. Other Funding: None.

20. Cancer Studies: Not applicable.

21. Biological Materials Transfer Agreement: No materials to be transported.

22. Investigational Drug Fact Sheet: No investigational drugs to be used.

23. Conflict of Interest: Form submitted here.

24. Nursing Staff: No impact.

25. Information Service: Will be completed.

030246
PENDING

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN RESEARCH PROTECTIONS PROGRAM

Date: March 7, 2003

To: Dr. William S. Cain Mailcode: 0957

Re: Project #030246
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

Although this protocol is a resubmission, the IRB takes each review opportunity to bring the protocols more closely in line with the current interpretation of regulations and research climate. Therefore, the Committee voted to approve this pending receipt of revisions and clarification:

1) The Committee had some difficulty determining what this was done on this study. For instance, the aims state that subjects have exposures of one-hour per day over four days. However, the consent states 12 sessions of up to 2.5 hours over 2-3 weeks. In addition, as this is an ongoing project, why was no information included about adverse events in phases I and II? How did subjects tolerate the exposures? How many terminated because of inability to tolerate chloropicrin? Please revise the application include this information and to clearly and specifically state what is currently being done on this study. Please review the consent to ensure that it reflects what is currently being done on the study and revise as needed.

2) The application and consent include that women will be excluded who are pregnant at the time of testing. Is there any possibility of long-term effects of WS3 that may affect women of child-bearing potential? Please clarify. Please revise the application and/or consent to address this issue. In addition, please revise the consent to more closely conform to wording outlined in the Human Research Protections Program fact sheet "Policy on the Use of Women of Child-Bearing Potential in Drug Studies" (enclosed).

3) Please revise the application, item 16, Privileges/Certifications and licenses, to include information about one of the co-investigators, Dr. Rolando Schmidt.

4) Please provide 700-U forms Statement of Economic Interest, for all co-investigators. Should any of these forms indicate that there is a conflict of interest (COI) with the sponsor, a revised consent that discloses the conflict to subjects is requested. Examples of this disclosure are: "Dr. X owns stock in (company's name), the sponsor of this research study;" or "Dr. X serves as a paid consultant for (company's name), the sponsor of this research study." In addition, the PI is

requested to forward a copy of the COI concurrence letter received from the COI office.

5) The consent states that a subject may withdraw from the study. Please clarify the procedures should the subject wish to withdraw once they have been placed in the study chamber. Please revise the application to include these procedures. Please revise the consent, as needed.
6) Please revise the consent to include wording that subjects will be informed of any significant research findings relevant to their continued participation.
7) Additional minor revisions to the consent as suggested.

Please send your reply along with two copies of the revised application and consent (one clean copy and one copy **underlining or bolding** the changes) to the attention of: Human Research Protections Program Office, mail code 0052. Final approval will be forwarded just as soon as we can determine that your responses are satisfactory.

Sincerely,

D. Masys

/cc

Daniel Masys, M.D., Director
Human Research Protections Program
Phone: 858-455-5050
E-mail: hrpp@ucsd.edu

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to WS3 (Phase 3a)

William S. Cain, Ph.D. and associates from the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of feel and odor from a chemical called WS3. The chemical is used commonly to impart some sense of feel in personal products. The results are intended to provide information regarding certain testing procedures we will use in later studies on chemical feel.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately 12 sessions of up to 2.5 hours over a period of a 2-3 weeks.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to WS3, you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by-Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to make judgments about the presence of feel from WS3, to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests.

You will receive $15 per hour for your time in screening and in testing. If you compete all testing, we estimate that you will earn an amount in the vicinity of $450.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-

investigators. If such a reaction should occur after you have left, ~~we will have you~~ page Dr. Bruff directly (619-407-1606).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late or fail to show up for scheduled sessions.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 455-5050 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

The Human Research Protections Program

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

~~No personal identifiers will be released in any public document or report.~~ Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

The UCSD Institutional Review Board

By signing you indicate that you have read, ~~understand~~ and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. ~~You agree to participate in the research described above.~~ You ~~may keep~~ will be given a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights," to keep.

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

SMALL REVISIONS AS REQUESTED ON 3/21 CONTACT

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor (Phase 3b)

William S. Cain, Ph.D., and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, in subsequent sessions we will perform various tests of how your nose and eyes respond to one-hour exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in three six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all 6 days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15 per hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $700. There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and

painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, ~~we will have you~~ page Dr. Bruff directly (619-407-1606).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 455-5050 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

POLICY ON THE USE OF WOMEN OF CHILD-BEARING POTENTIAL IN DRUG STUDIES

1. If there is a known likelihood of risk to a fetus, the Human Subjects Committee may require that women of child-bearing potential be excluded from participation.

   If however, there is a potential for direct benefit to the subject or overwhelming benefit to society, the Committee may approve the inclusion of women of child-bearing potential with stipulations that there be appropriate screening, monitoring, discussion, and informed consent.

2. If the likelihood of harm to a fetus in not known, the Committee may require the principal investigator to provide assurance (by pregnancy test or other appropriate criteria) that the women participating are not pregnant and instructions (via the consent process) on the necessity for the use of effective contraceptive measures if the study continues over a period of time.

Suggested Language For Consent Forms When Women of Child-Bearing Potential Are Involved.

1. *If you are a female and capable of child-bearing, a sample of urine will be collected before the study is begun in order to be as sure as possible that you are not pregnant. It is important to be as sure as possible that you are not pregnant since the ... being tested may cause harm to an unborn child.*

   or

2. *If you are a female and capable of child-bearing, a sample of urine will be collected before the study is begun in order to be as sure as possible that you are not pregnant. Your participation requires that you use a birth control method, such as... to prevent pregnancy during the study, as the ... being tested may cause harm to an unborn child.*

3. *If you are female and capable of child-bearing, a sample of urine will be collected before the study is begun in order to be sure as possible that you are not pregnant. Your participation requires that you use contraception methods (such as abstinence, diaphragm, condom, or an intrauterine device) to prevent pregnancy for the duration of the study, as use ... being tested may cause harm to an unborn child. If you miss a period or think you might be pregnant, you will notify the doctor. You may have to withdraw from the study.*

UCSD Human Researc
Biomedical Project: Standa
This form can be used for paper-based submissior
Please follow the instructions tha

HSC Proj Nr: 030246 Mtg: 03/06/2003
"Human Sensory Irritation Testing for Chloropicrin"
P.I.: William Cain, Ph.D
Primary: Wallace Second: Heldt

03

Instructions for submitting on paper:	Instructions for submitting electronically:
1. Complete all 3 Pages of this form. Use Acrobat Reader to fill in this form (preferred) or print or type legibly. 2. The Principal Investigator, Department Chair and for VA projects, the Service Chief must sign where indicated on the last page. 3. Attach these facesheets to the completed **Research Plan**, consents, and other documents associated with the project. Submit **20 printed copies** of all materials to the UCSD HRPP office, mail code 0052. The template for the Research Plan can be downloaded from http://irb.ucsd.edu in MS Word format. 4. For sponsored clinical trials, include two copies of the Master Protocol and Investigator's Brochure.	1. Complete all 3 Pages of this form. **To do this, open the form using your Web Browser** to fill in the form (requires Acrobat Reader or plug- in). 2. Click the PRINT button on the last page to make a copy for signatures. 3. Click the Submit button on the last page to submit the data from the facesheets to the HRPP office via the Internet. 4. The Principal Investigator, Department Chair and for VA projects, the Service Chief must sign where indicated on the last page. 5. Mail one copy of the signed facesheets to UCSD HRPP Office, mail code 0052. 6. Upload the accompanying **Research Plan,** consents and other documents to the http://irb.ucsd.edu website. The template for the Project Plan is available on the website in MS Word format.

Section 1: PROJECT TITLE*

Human Sensory Irritation Testing for Chloropicrin

*For sponsored projects include sponsor's project identifier and version number. For VA merit grants, title must match the grant title

Section 2: KEY PERSONNEL

Principal Investigator	Last name	Cain	First Name	William	Degree*	Ph.D.
	Title	Professor	Department	Surgery/Oto	Mail code	0957
	E-mail	wcain@ucsd.edu	Phone	858-622-5831	Fax	858-458-8417
	Principal Investigator is salaried UCSD or VASD employee (check Yes or No) Yes	✓	No			
Contact	Last name	Same	First Name		Degree*	
	Title		Department		Mail code	
	E-mail		Phone		Fax	

* Degree = Ph.D., M.D., R.N., etc.

Section 3: PROJECT CHARACTERISTICS

Yes	No	
✓		This is a **renewal of a previous project.** If Yes: The IRB number for the previous project is 010201 and the participant accrual to date at this site is 126. Total projected participant accrual for the entire project:
Yes	No	
	✓	Will recruit participants **under age 18** (Note: if study being done at VA facilities, must have VACO waiver)
✓		Will recruit **women of child-bearing potential**
	✓	Will recruit **pregnant women**
	✓	Will recruit **cognitively-impaired individuals**
	✓	Will recruit **prisoners**
	✓	Will recruit patients with **cancer**
	✓	Will recruit patients at **VA Medical Center**
	✓	Involves **gene therapy**
	✓	Involves **human fetus or fetal tissue**
	✓	Involves **waiver of consent** (i.e. the research will be done without seeking the consent of persons whose records or tissue are analyzed)
	✓	Involves **waiver of documented consent** (ie., consent obtained but there is no signed consent form)
	✓	Involves **banking of tissue or fluids**
	✓	Involves **DNA genotyping or other form of genetic analysis**
	✓	Has a **Data and Safety Monitoring (DSM) Board** or DSM Plan

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Section 4: INVESTIGATIONAL DRUGS, DEVICES AND PROCEDURES

Yes	No					
	✓	Involves FDA **Investigational New Drug** Application(s)				
		If Yes, enter the following:				
		Investigational **drug name(s)**, one drug name **and associated IND number** per line.	Drug name(s):	1. 2. 3.	and IND number(s):	1. 2. 3.
			Project will use UCSD Medical Center Investigational Drug Service:		Yes: ☐	No: ☐
		If the Investigational Drug Service **will not be used**, enter the following:	**Location** where drugs will be stored:			
			Name of person responsible for dispensing study drug(s):			
			Phone number of person responsible for dispensing study drug(s):			
	✓	Involves FDA **Investigational Device Exemption(s)** or FDA **Category B device**				
		If Yes, enter device name(s), one device name and associated FDA-assigned IDE, 510(k) or Category B identifier per line:	Device name(s):	1. 2. 3.	and FDA number(s):	1. 2. 3.
	✓	Study participants will be exposed to **Radiation or Radioactivity**				
		If Yes, enter the following about the sources of radiation:	Radiographic **X-ray**		Yes ☐	No ☐
			Fluoroscopy		Yes ☐	No ☐
			DEXA (Bone Density)		Yes ☐	No ☐
			Computed Tomography (CT)		Yes ☐	No ☐
			Positron Emission Tomography (PET)		Yes ☐	No ☐
			Nuclear Med. **(radionuclide)** injections		Yes ☐	No ☐
			If Yes, Name(s) of **Nuclear Medicine Procedure(s)**			
			A non-routine radioactive drug		Yes: ☐	No: ☐
			If Yes, enter Radioisotope Use Authorization (RUA)*			
			Other form of radiation or radioactivity		Yes: ☐	No: ☐
			If Yes, describe			

*Projects with this type of Radioisotope use must complete RDRC application available from Radiation Safety Office.

Section 5: FACILITIES WHERE STUDY WILL BE CONDUCTED

Yes	No	
	✓	UCSD Healthcare hospitals or clinics
	✓	UCSD General Clinical Research Center (GCRC)
	✓	VA San Diego Healthcare System hospital or clinics
	✓	San Diego Children's hospital or clinics
✓		Other: Describe facilities here: Chemosensory Perception Lab, La Jolla Village Prof Center

Section 6: FUNDING

Funding Source Type (check all that apply)	☐	Unfunded	✓	Commercial sponsor	☐	Departmental or ORU funding
	☐	Federal agency	☐	Academic Senate	☐	VMRF administered
	☐	Not for profit foundation	☐	VA funded		
Funding Mechanism	☐	Grant	☐	Gift	☐	OCGA Research Agreement (RA)
	✓	Contract	☐	Internal		
	☐	Clinical Trial Agreement	☐	Other funding mechanism - specify:		
Funding Status	✓	Awarded	☐	Pending		
Sponsor Name						

Other project Identifiers	UCSD OCGA proposal number		Sponsor's ID (e.g, NIH grant Nr)	
	SOM Clinical Trial agreement number		Investigator-initiated or RA number	
	VA PDS number			

Fiscal Contact	Last Name	Fletcher	First Name	Jody	Department	Surgery
	E-mail	jfletcher@ucsd.edu	Phone	619-543-3109	Fax	

Continued next page

Section 7: OTHER PERSONS ASSOCIATED WITH THIS PROJECT					
Role	Last name	First name	Degree	Department	Institution
Co-Investigator	Jalowayski	Alfredo	Ph.D.	Surgery/Pediatricss	UCSD
Co-Investigator	Davidson	Terence	M.D.	Surgery	UCSD
Co-Investigator	Bruff	Thomas	M.D., M.P.H.	Medicine	Private Prac/UCSD
Co-Investigator	Schmidt	Roland	Ph.D.	Surgery	UCSD
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Co-Investigator					
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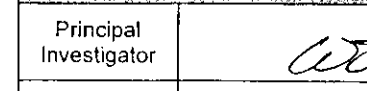
Co-Investigator					
Co-Investigator					
Other role:					
Other role:					
Other role:					
Other role:					

Section 8: Signatures			
Principal Investigator		Date:	2/20/03
Department Chair		Date:	
Service Chief (for VA projects)		Date:	

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030246

UCSD Human Research Protections Program

Biomedical Project: Standard Application for Review

This form can be used for paper-based submissions to the IRB and also for electronic submissions.
Please follow the instructions that apply to your submission type:

Instructions for submitting on paper	Instructions for submitting electronically
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FEB 2 6 2003

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			Nuclear Med. (**radionuclide**) injections		Yes ☐	No ☐
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Fiscal Contact						
Last Name	Fletcher	First Name	Jody	Department	Surgery
E-mail	jfletcher@ucsd.edu	Phone	619-543-3109	Fax	

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Co-Investigator	Davidson	Terence	M.D.	Surgery	UCSD
Co-Investigator	Bruff	Thomas	M.D., M.P.H.	Medicine	Private Prac/UCSD
Co-Investigator	Schmidt	Roland	Ph.D.	Surgery	UCSD
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Co-Investigator					
Other role:					
Other role:					
Other role:					
Other role:					

Section 8: Signatures

Principal Investigator		Date:	2/20/03
Department Chair		Date:	2/21/03
Service Chief (for VA projects)		Date:	

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FINAL NOTICE

Project #: 010201

YOUR RESPONSE IS DUE NOT LATER THAN: 2/20/2003

Date: January 29, 2003 Rec'd 2/20 copies ss revb.

To: Dr. William S. Cain Mailcode: 0957

From: HUMAN RESEARCH PROTECTIONS PROGRAM (HRPP)

Re: Human Sensory Irritation Testing for Chloropicrin

The DHHS, the FDA and the University of California regulations REQUIRE that all investigations involving human subjects have at least an annual review. Please answer the following questions.

1. Has the project been activated? Yes ✓ No___
   If yes, is it active now? Yes ✓ No___

   If project is **NOT** active, please indicate why:
   a. Study has been terminated by sponsor_____
   b. Study is closed to accural, with non-research activity follow-up_____
   c. Study is completed, but data analysis portion not complete_____
   d. Other_____

If this project involves direction interaction or intervention with subjects, please answer all remaining questions. If not, skip to #4.

2. How many subjects have been studied to date at UCSD sites? 126 at all sites?___

3. Have there been any injuries to subjects or any unexpected reactions that involve risk to subjects or others? Yes __ No ✓, though see accompanying file regarding one subject

   If yes, and you have not yet notified the HRPP of these problems, a detailed description of your experiences should be returned with this notice.

4. **Approval for this project will expire on 3/7/2003.**

If you plan to continue this research please complete and return this form, a new and updated application, including unstamped consents, for review. ***A summary of experience must be included with the updated research plan for resubmission.*** Appropriate forms are available on the HRPP website at irb.ucsd.edu. If you have questions about this, please call the HRPP office at 858-455-5050. You may submit your research plan to HRPP at mail code 0052 or upload it at irb.ucsd.edu, e-irb. (Please note that the e-irb meets only once a month.)

WSCain — Signature of Principal Investigator

0957 — Mail Code

2/19/03 — Date

658-622-5831 — Phone

Title of Project: Human Sensory Irritation Testing for Chloropicrin

Principal Investigator: William S. Cain, Ph.D., Professor of Surgery (Otolaryngology)

Co-Investigators: Alfredo Jalowayski, Ph.D., Research Specialist, Depts. of Pediatrics and Surgery (Otolaryngology), Terence M. Davidson, M.D., Professor of Surgery (Otolaryngology-HNS), Thomas Bruff, M.D., M.P.H., Assistant Clinical Professor of Medicine, and Roland Schmidt, Ph.D., Postgraduate Researcher, Dept. of Surgery (Otolaryngology)

1. Facilities: The work will be performed in the Chemosensory Perception Laboratory at the La Jolla Village Professional Center.

2. Duration: One to two years.

3. Specific Aims: There are three phases of work, each with a specific aim, as follows:

Aim 1: To establish the sensitivity of olfaction, chemesthesis (i.e., feel or irritation) of the nose, and chemesthesis of the eyes for momentary exposures to chloropicrin directed to the sites. **(Completed.)**

Aim 2: To establish sensitivity for ambient exposures where all channels for detection are available and, within this framework, to establish whether sensitivity varies among exposures of different durations, ranging from a few seconds to 30 minutes. Of particular interest with respect to time-course is whether undetectable concentrations become detectable over time. **(Completed.)**

Aim 3: To establish whether mildly irritating ambient exposures of one-hour per day over four days lead to a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue.

Aims 1 and 2 pertained to all persons, those exposed adventitiously from environmental releases of chloropicrin and those exposed occupationally. Aim 3 pertains primarily to persons exposed occupationally and more likely to have repetitive exposure. Aim 3 can also apply to residential exposure because off-gassing may occur for several days from a single field. If there are multiple fields in an area, residents could conceivably be exposed intermittently for a longer time.

4. Background and Significance

Chloropicrin is a colorless liquid with a sharp, penetrating odor. The material is used primarily to fumigate fields in order to control soil-borne fungi, plant diseases, and nematodes. Pre-planting soil fumigation with chloropicrin makes it possible for plants such as strawberries to achieve exceptional root-growth unaffected by soil-pests and disease. Because of the sharpness of its vapors, chloropicrin has also been added to other, odorless fumigants, such as sulfuryl flouride and methyl bromide as a warning

agent. The same property of sharpness has led to use of the material as an agent to test the vapor attenuating properties of activated carbon and the fit of personal protective respiratory devices.

The mammalian acute and chronic toxicology of chloropicrin, including that following inhalation, is well described and current. Testing in laboratory animals by inhalation or other routes of exposure has indicated that chloropicrin in nonsystemically toxic exposures poses no hazard to pregnancy or to the developing fetus, nor does it impair reproductive functioning. It has been found to be noncarcinogenic following lifetime inhalation exposure to rats and mice and in chronic feeding studies to beagle dogs. A chronic oral (gavage) study in rats produced a single animal with a stomach papilloma in the test group receiving 10mg/kg/day, the highest dose tested. This papilloma is considered to be spontaneous in origin. There is no evidence that chloropicrin will bioaccumulate in mammalian cells.

Human beings come into contact with chloropicrin principally on the job in agriculture or, to a lesser extent, in wood preservation. The American Conference of Governmental Industrial Hygienists (ACGIH) initially set the threshold limit value (TLV) at 1 ppm (time-weighted average, TWA) in 1957 and reduced it to 0.1 ppm in 1959, where it remains today. In the documentation, ACGIH states: "A TLV-TWA of 0.1 ppm is recommended for repeated exposure to chloropicrin to prevent eye irritation and the potential for pulmonary changes" (p. 299). OSHA in the U.S. and its regulatory counterparts in many industrial countries also use a TWA of 0.1 ppm as the permissible exposure limit.

The repellent properties of chloropicrin at low levels include reflex blepharospasm, tearing, and pungency that can effectively function as warning properties. As Krieger (1996) noted in an exposure and risk assessment: "This protective reflex response is an important homeostatic mechanism by which exposure is reduced and the sensitive human pulmonary system is spared adverse effects resulting from higher levels of chloropicrin. ...It is a protective reflex response that occurs when chloropicrin contacts the trigeminal nerves of the moist nasal airways. Tearing (lachrymation) and painful stinging eyes results from temporary disturbances of the eye that are completely reversible and occur at concentrations of 0.15 ppm to 0.3 ppm chloropicrin. This undeniable reflex response warns persons to move to uncontaminated environments before toxic exposures occur." (Pp. 10-11.)

Krieger noted further that the mandated use of chloropicrin to warn of the presence of odorless fumigants "gives clear evidence of regulatory recognition of the importance and usefulness of the warning properties of low levels of chloropicrin" (p. 12). To illustrate, U. S. EPA PR Notice 84-5 describes the language to be used for methyl bromide that contains chloropicrin: "This product contains chloropicrin as a warning odorant. Chloropicrin may be irritating to the upper respiratory tract, and even at low levels can cause painful irritation to the eyes, producing tearing. If these symptoms occur, leave the fumigation area immediately." (U. S. EPA, 1984; p. A-1).

The table below, from Krieger (1996), shows human responses to airborne chloropicrin at various levels and at exposures from instantaneous to chronic to the extent that these have been studied. In the main, the effects derive from direct contact between chloropicrin and tissue at the portal of entry. The nausea and vomiting that may occur from high levels of exposure are thought to occur because of swallowing of the irritating material that has dissolved into saliva.

Until the present investigation, human responses to airborne chloropicrin have been known mainly through anecdotal reports or from studies and other observations collected many decades ago. These experiences indicated that exposure to airborne chloropicrin concentrations of about 0.15 ppm to 0.3 ppm can cause tearing and eye irritation that is reversible upon cessation of exposure.

The results of these studies will provide an appropriate framework to learn whether odor or sensory irritation that signals low-level exposure to chloropicrin provides an adequate mechanism to prevent occurrence of adverse effects seen in high-dose animal studies. This work also will allow evaluation of important human attributes of the responses to low-level chloropicrin; individual variability in responses; differences in sensitivity as a function of modality of response (ocular stimulation vs, nasal stimulation); and repeated vs. single exposure responses that may be important manifestations of the sensory response to chloropicrin.

Together, the information from these studies will be useful not only in human hazard assessment, but also for the promulgation of exposure standards for workers and the general population.

This research plan was devised in response to a request for proposals from the Chloropicrin Manufacturers Task Force. The protocol was, however, created by the investigators at UCSD.

Human Responses to Airborne Chloropicrin Exposures[1]

Exposures	Conc. x Time	Response	Characteristics
Workplace or Off-Site	Less than TLV-TWA 0.1 ppm or less	None No Adverse effects (eye): NOAEL	None
Instantaneous Contact	0.15 to 0.3 ppm x secs	*Common* Chemical *Sense Threshold*	Tolerable *with very slight or no irritating sensation*
		Reflex tearing and reflex coughing	① Concentration dependent
			②Response ceases when exposure removed
			③Response non-cumulative
			④Primary irritant, little or no systemic effect
	TLV-TWA: 0.1 ppm or less		
Acute	Greater than TLV-TWA of 0.1 ppm; seconds-minutes	Irritation	*Slightly irritating, some stinging or burning of eye, nose, throat*
	0.15-0.3 ppm; 3.30 secs.	Lacrimation	
	0.3-0.37 ppm; 2-2.5 seconds	Eye irritation; LOAEL	
	0.9 ppm	Odor threshold	
	1.5 ppm; few seconds		⑤ Secondary airway irritant; lacrimation, nausea, vomiting
	4 ppm; few seconds	Pulmonary Toxicity	Upper respiratory tract irritation; tissue injury; edema
Chronic	0.1 ppm	No Effect	None

[1]To the best of our knowledge, no controlled human studies of chloropicrin exposure have been completed. Each of these values in this table is anecdotal or derives from a source for which analytical verification of chloropicrin concentrations and standardized evaluation of subject response does dot exist. The present protocol describes a laboratory study that incorporates proper and comprehensive control of variables as well as appropriate analytical and psychophysical response measurement technology to assure valid results having the greatest degree of scientific certainty..

5. Progress

We have collected the data regarding aims 1 (Phase 1) and 2 (Phase 2), but have not yet finished the analysis. The total number of participants who passed screening equaled 126, half males and half females. Almost all of these persons participated in exposures in Phase 1, Phase 2, or both. A small number of people did not go on to either phase, an occurrence we see in every study that entails some time between screening and further participation.

Phase 1 focused upon determination of the threshold for odor, for feel in the eyes, and feel in the nose (see Figure 1 for the subject's task of choosing which of three cones at a station had odor). The threshold odor equaled 750 ppb, averaged over the 63 participants in this phase. The threshold for feel in the eyes equaled 929 ppb (30-sec exposure). And, the threshold for feel in the nose proved indeterminate over the range of concentrations explored. It was therefore clear from the testing that for the average subject, the eyes were more sensitive. The threshold for odor proved to be a little lower than previous estimates and the threshold for feel in the eyes proved to be somewhat higher. Nevertheless, as Phase 2 indicated, the threshold for feel in the eyes is quite time-dependent. We obtained enough data from each subject to make meaningful comparisons of the individual differences that underlie the average threshold. This will be an important part of our analysis over the next months.

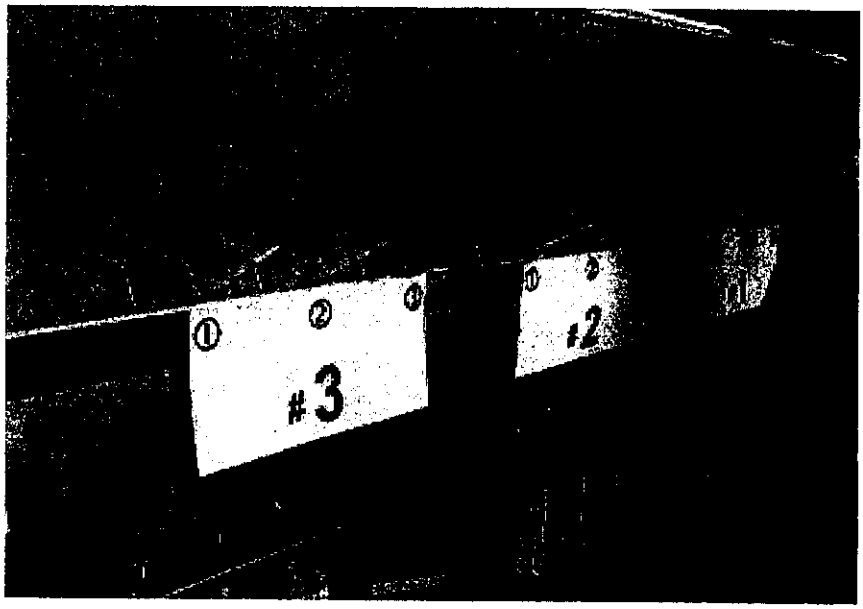


Figure 1. Showing a subject interacting with the delivery device used to present vapors for short exposures (2 sec for odor and nasal feel; 30 sec for feel in the eyes). A computer controlled voice guides the subject through the task.

In Phase 2, a total of 62 subjects sought to detect the presence of feel from chloropicrin in the environment of a chamber and to rate confidence in their judgments of detect-no detect (see Fig. 2). In some exposures chloropicrin was present and in some it

was not. (The levels were too low for the subjects to detect any odor.) In this phase we found that over time subjects could detect feel in the eyes at much lower concentrations than the 929 ppb felt in Phase 1. Subjects could, for example, eventually feel a concentration of 75 ppb after many minutes of exposure. Demonstration of the time-dependence of detection of irritation is important new information regarding chloropicrin. As in the testing in Phase 1, individual differences in sensitivity will be a focus of analysis.

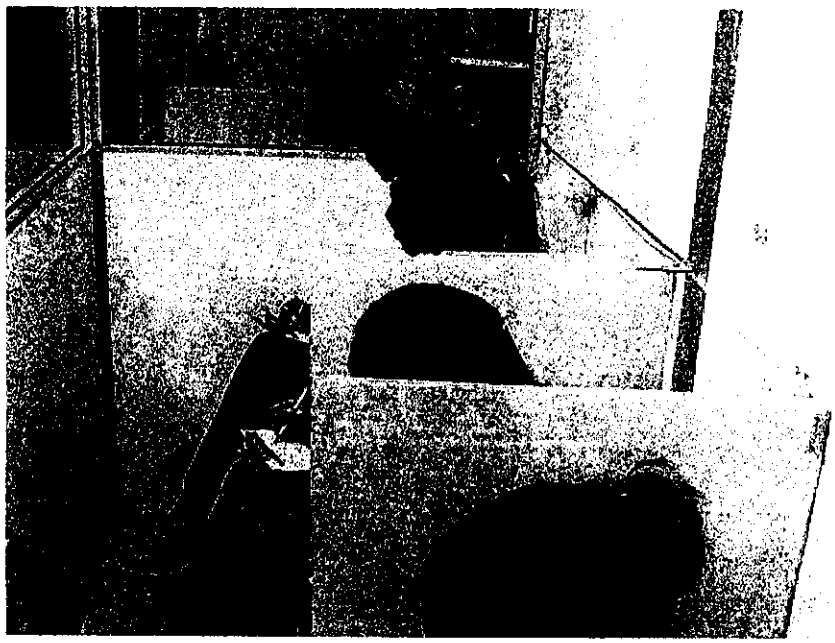


Figure 2. Subjects in the setting for Phase 2, where they sought to detect presence of chloropicrin over time. They made judgments every minute for 20-30 minute sessions. This setting will also be used for the experiments of Phase 3.

As we analyze the results of Phases 1 and 2, we are prepared to move into Phase 3.

Phase 3: Signs/Symptoms of Irritation in Daily Exposures

Objective: To establish whether mildly irritating ambient exposures of one hour per day over four days lead to: a) evidence of discomfort that lasts materially beyond the periods of exposure and b) evidence of inflammatory changes in the mucosal tissue. The level of exposure will be designed to be low enough to induce no inflammatory effect in the lower airways.

The objective will be addressed in two stages, Phase 3a and Phase 3b.

Phase 3a - Preliminary Study to Validate Techniques of Measurement: This phase will serve to establish: a) sensitivity and stability of clinical assessment of signs and symptoms, b) sensitivity and stability of three assays for biochemical markers, and c) sensitivity and stability of the Rhinconjunctivitis Quality of Life Questionnaire in the

context of the present work (e.g., Juniper, Thompson, Ferrie, & Roberts, 2000). Statistically speaking, the preliminary study deals with issues of the power to detect effects of exposure to chloropicrin when these exist, but the preliminary study of Phase 3a will entail no exposure to chloropicrin. It will instead set the stage for exposure to chloropicrin in Phase 3b.

Clinical Studies of Rhinitis: In some respects, the agenda for Phase 3 resembles that of clinical studies of effects of medication on allergic rhinitis. In view of the uniqueness of the present work, the similarities to and differences from those clinical studies seem worthy of comment. In such studies, investigators may challenge subjects with allergens in order to induce reactions and may measure how medication diminishes sensitivity or reactivity. Outcome variables of interest generally include a score for signs/symptoms and perhaps an index of quality of life, such as the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), and some objective indices, such as biochemical markers of inflammation. Variables of this nature have relevance to the present research. In the present case, however, a major question concerns whether subjects will become symptomatic and show signs of rhinitis from environmentally plausible exposures rather than whether they will become less symptomatic from medication. If they become symptomatic, then the outcome variables need to show the result and its various manifestations with clarity. If they do not, then the investigators need to show that the outcome variables would have registered a positive result if it had occurred. This accounts for concerns over sensitivity and stability.

Blinding and a "Reverse Placebo:" The present study differs from clinical trials not only in that the agent of interest may induce symptoms, but also in: a) the inability to blind subjects to presence of the agent, a noxious vapor, b) absence of a reliable way to provoke rhinitis in normals and reluctance to do so needlessly, and c) the time-frame of the effects. In the clinical trials, investigators normally compare subjects treated with the medication, these days typically a topical agent, against those given a placebo. In some instances, such as some studies of seasonal allergic rhinitis, the investigators may not provoke symptoms in the laboratory but may rely upon everyday exposures to keep the subjects symptomatic. In those cases, the investigators gather data before treatment and compare it with data gathered during a regimen of treatment. Even though the studies have the opportunity to use subjects as their own controls, the studies will normally include two groups, one treated with active ingredient and one given placebo. A greater reduction in signs/symptoms in the group treated with active ingredient counts as success (e.g., Van Cauwenberge, Juniper, & the STAR Investigating Group, 2000). In such studies, the subjects who receive the placebo commonly show some subjective improvement, i.e., reduction in symptoms (see Kobayashi, Beaucher, Koepke, Luskin, et al., 1995; Meltzer, Jalowayski, Orgel, & Harris, 1998). If the studies measured effects before vs. after the medication only, the outcome would inflate the benefits of the medication. In the same manner, studies of exposure to an irritating vapor may inflate symptoms unless they use the equivalent of a "placebo." At this point, we can only speculate about whether this will prove true.

Whereas investigators in the clinical trial can blind subjects to the presence or absence of the active ingredient, investigators of a noxious exposure cannot. As an

approximation, though, the investigators can expose subjects to a vapor that precipitates no actual irritation, but at least stimulates the same mucosal tissue as an irritating vapor. In the present case, we have chosen a material known as WS 3, a non-irritating, odorless cooling agent used in consumer products, as this type of "reverse placebo." Its use in Phase 3a will serve to examine the lability of subjects' symptoms as part of the investigation of sensitivity and stability of the ratings of symptoms.

Positive Control Group vs. Positive Control Exposure: Clinical studies provoke symptoms and signs in allergic subjects under conditions well characterized with respect to agent and duration of effect. Hence, when an investigator sprays a dilute solution of ragweed pollen extract into the nose of a person with known allergy to that substance, he can anticipate that an ensuing acute episode may begin within minutes and subside after an hour. The protocol may even entail increasing the concentration of the challenging agent until a response occurs. This establishes the positive control. The study of a noxious vapor cannot easily follow this simple route. It is unclear how to provoke rhinitis in a normal person. An irritating vapor may do so, but at this time we know neither the vapor nor the level that will do so unfailingly. Nor can we know how to provoke the symptoms as temporarily as one might in persons with allergic rhinitis. How then can one demonstrate the sensitivity to resolve the presence vs. the absence of rhinitis in the study of a noxious vapor? One way would be to demonstrate the ability of the outcome measurements to resolve between normals and persons already symptomatic. For this, one can study persons screened for normal nasal health and persons screened for presence of symptomatic allergic rhinitis. By this device, a positive control group substitutes for a positive control exposure.

Ratings: In clinical studies of rhinitis, investigators rather commonly ask subjects to fill out simple ratings of symptoms such as that shown in Table 1 below. The scale reportedly picks up differences of approximately half a step in nasal score with power greater than 90% in groups of a dozen or so subjects, but the published literature contains little documentation of such sensitivity (Meltzer, personal communication).

Although indispensable in clinical studies, ratings of symptoms fail to capture the effects of symptoms on everyday life. This situation has given rise to questionnaires that assess quality of life. The RQLQ, a self-administered questionnaire of high reliability (Cronbach's alpha >0.90) and validity, assesses quality of life as pertains to the nose and eyes. Via a series of 28 questions it assesses how "nose/eye symptoms trouble you in your life." Regarding sleep, for instance, it inquires: "How troubled have you been by each of these sleep problems during the last week as a result of your nose/eye symptoms? 4. Difficulty getting to sleep. [The respondent has six choices per item ranging from *not troubled* to *extremely troubled*] 5. Wake up during night. 6. Lack of a good night's sleep." Most commonly, ratings of quality of life ask the respondent to consider the previous six or seven days. In general, the RQLQ is not given on sequential days, but we see no a prioi reason why it would not work well even though many days of the frame of reference, a week, would overlap.

Clinical studies of rhinitis include ratings from clinicians as well as ratings from subjects. Although the ratings of the clinicians include signs, such as congestion of the

nose and tearing of the eyes, they also include the impressions of the subjects. Consequently, it hardly surprises that the ratings of the subjects and the ratings of the clinicians agree extremely well (see Meltzer et al., 1998). In a study that cannot blind the subject from the nature of an exposure, it seems essential to blind the clinician. The clinician can use a rating scale not unlike that of the subject (), but should remain oblivious to conditions of exposure to remain unbiased.

Biochemical Markers: Irrespective of whether it arises from allergies, infection, or irritation, rhinitis represents an inflammation of the nasal mucosa. The presence of inflammation reveals itself via the type and number of cells that can be sampled from the mucosa. In samples taken by Rhinoprobe and quantified as number of cells per high power field (HPF), normal superficial nasal mucosa has the following cytologic profile: neutrophils 0-10.5, eosinophils 0-0.45, and basophilic cells 0-0.2 (Jalowayski & Zeiger, 1988). When neutrophils reach 16-20, eosinophils reach 1.1-5.0, and basophilic cells reach 0.4-1.0, subjects characteristically exhibit medically significant inflammation.

The presence of inflammation in mucosal tissue can also reveal itself via levels of biochemical markers. Those shown to increase significantly in nasal and conjunctival mucosa, nasal secretions, and tear fluid after challenge with allergens include albumin, interleukin-8 (IL-8) and soluble intercellular adhesion molecule (sICAM) (Ciprandi, Pronzato, Passalacqua, Ricca, et al., 1996; Calderon, Devalia, Prior, Sapsford, & Davies, 1997; Granstrand, Nylander-French, & Holmstreom, 1998; Wilson, Lau, & Howarth, 1998). Albumin leaks across the mucosal layer during an inflammatory process. Its concentration in nasal fluid and tears collected via absorbent sponge can be measured by ELISA. IL-8, one of several proinflammatory cytokines that play a major role in attracting and activating inflammatory cells, can also be measured by ELISA. sICAM, known to play a role in eosinophil and neutrophil infiltration across endothelial and epithelial cells, can also be measured by ELISA.

The accuracy of research on markers in secretions can depend upon the mode of collection, a constantly evolving matter as investigators abandon error-prone methods. The present research will entail collection of fluid with small cellulose sponges. Such collection does not irritate and avoids uncertainties regarding dilution as in the method of nasal lavage. Weight of the sponge before and after 30-sec application to the mucosa gives an exact measure of amount of fluid collected. The sponge also elutes albumin better than filter paper, another medium of collection. Because collection via the sponge is new, few data address sensitivity to analytes except for eosinophilic cationic protein (ECP) and tryptase in secretions (Klimek, Wolf, Mewes, Dormann, et al., 1999). We believe that we will collect sufficient quantities of nasal and tear fluid to measure levels of albumin, IL-8, and sICAM accurately. The preliminary study will test ability to resolve between normals and symptomatic persons.

Preview of the Preliminary Study: The study described in its particulars below will entail the following: Two groups of subjects, one screened for absence of nasal inflammation and the other for presence of allergic rhinitis will participate in eight one-hour sessions, a block of four on successive days that will entail exposure to air and another block of four on successive days that will entail exposure to a cooling level of

WS 3. Half the subjects per category will have the exposure to air first and half the exposure to WS 3 first. In neither case, should the subjects experience irritation from the stimulus. Subjects will have a clinical exam of the nose, eyes, and throat before each exposure and twice after the exposure, within a half-hour and after another hour. At the approximate times when subjects will have the clinical exams, they will also rate their symptoms. In connection with the clinical exams, the examiner will collect fluid from the nose and eyes for analysis of the markers albumin, IL-8, and sICAM. Before an exposure and 24 hours after the fourth exposure in a block, the subject will fill out the RQLQ with respect to the previous 24 hours.

Questions addressed in the preliminary study will include:

1) How well will a blinded clinical exam resolve between the normals and the subjects with rhinitis? We expect that the exam will resolve between the groups, but the variability day to day, across groups, and across exposures should yield important statistical information about the sensitivity and stability of the exam.

2) How much lability will subjects show in their ratings of symptoms? Will the ratings differ between exposures to air and exposures to cooling agent? The answer may have implications for how to control against biased ratings in studies that cannot blind subjects to exposures. The study will also, however, offer statistical information about the sensitivity and stability of the particular ratings of symptoms (Table 1). How, one might ask, can we know that the cooling agent does not cause irritation and that any increase in symptoms does not occur because of actual irritation? The clinical exam can serve as arbiter here. Moreover, quite possibly the subjects with allergic rhinitis might have their symptoms reduced by exposure to the cooling agent.

3) Will the RQLQ perform meaningfully for resolution between normals and persons with rhinitis when given repeatedly?

4) Will secretions collected with sponges provide enough material for reliable and sensitive assays for the markers albumin, IL-8, and sICAM?

Stated as an objective, Phase 3a entails the following:

Objective: To establish in 32 subjects (16 males and 16 females), half of them (eight males and eight females) screened for normal mucosal condition and the other half for allergic rhinitis, the stability and sensitivity of various tests to reflect the presence of mucosal inflammation.

Table 1

Rating of Symptoms

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Collected by:_____

Use the following scale to indicate the degree of symptoms:

Scale	Degree	Meaning
0	None	No symptom.
1	Mild	Symptom present, but minimal awareness, easily tolerated.
2	Moderate	Symptom definite and bothersome, but tolerated.
3	Severe	Symptom hard to tolerate and can interfere with activities of daily living or sleeping.

Nose		
a. Congestion	____	
b. Runny Nose	____	
c. Itchiness/Sneezing	____	
d. Irritation	____	____
Eye		
a. Tearing	____	
b. Puffiness	____	
c. Itchiness	____	
d. Irritation	____	____
Throat		
a. Cough	____	
b. Hoarseness	____	
c. Dryness	____	
d. Irritation	____	____
	Total Score	____

Table 2

Rating of Signs

Subject ID Number:_____ Code:_____
Date:_____ Time:_____
Examined by:_____

Use the following scale to indicate the degree of clinical signs:

Scale	Degree
0	No sign evident.
1	Sign barely present.
2	Sign clearly present.
3	Sign quite marked.

Nose
a. Congestion ____
b. Rhinorrhea ____
c. Erythema ____ ____

Eye
a. Tearing ____
b. Puffiness ____
c. Erythema ____ ____

Throat
a. Cough ____
b. Swelling ____
c. Erythema ____ ____

Total Score ____

Apparatus: Exposures will take place in the chambers, with concentration of WS 3 (n-ethyl-5-methyl-2-(1-methylethyl)-cyclohexanecarboxamide, CAS 39711-79-0) controlled in the manner used for Phase 2, i.e., vaporization of liquid injected onto a warmed surface. Other apparatus will include that used in screening (see details under **7. Human Subjects**).

Procedure: Screening will establish two groups of subjects, 16 with and 16 without evidence of nasal inflammation (see details under **7. Human Subjects**). Every subject will participate in the same tests that will revolve around eight exposures of one-hour in the chambers. Four exposures will take place in one block of four days and four in another block, with a minimum four-day break between blocks. In one span of four days, subjects will have exposure to WS 3 at a just-cooling level and, in the other span, those same subjects will have exposure to just air on the four successive days. Half the subjects will have exposure to WS 3 first and half exposure to air first.

On each day of exposure, the following will be performed before exposure begins (details of exams appears under **7. Human Subjects**):

1) subject fills out the RQLQ,

2) subject rates symptoms (see Table 1),

3) clinical examination of the nose, eyes, and throat, and rating of signs (see Table 2),

4) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, and

5) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min.

The following will then be performed in the chamber:

1) subject enters the chamber for an hour, and

2) in the last half-minute of exposure, the subject rates symptoms.

Fifteen min after exposure, the following will be performed:

1) subject rates symptoms,

2) clinical examination of the nose, eyes, and throat, and rating of signs,

3) sample of nasal secretion taken, as before exposure, and

4) sample of tear fluid taken, as before exposure.

Procedure: Subjects will serve in three blocks of six sessions, each beginning on a Friday and ending on a Friday. Exposures to a given concentration in the chambers will last one hour on four successive days, Monday through Thursday. In one block, a subject will have exposure to chloropicrin at 0.1 ppm. In another block, the subject will have exposure to 0.15 ppm, and, in a third block, the subject will have exposure to just air on the four days. The order in which a given subject has exposure to the three conditions will vary to prevent confounding of order of exposure with level. Except for personnel who set and monitor the conditions in the chamber, the personnel who will deal with the subjects and the subjects themselves will be blinded to the conditions at any given time. At least one week will separate the end of one block and the beginning of another for a subject.

On the first Friday of a block, baseline measures will be taken (see Table 3). The following will be performed:

1) a Rhinoprobe sample taken from the inferior turbinate in one nostril to establish the number and kind of cells in the mucosal layer (see details under **7. Human Subjects**),

2) subject fills out the RQLQ,

3) subject rates symptoms (see Table 1),

4) clinical examination of the nose, eyes, and throat, and rating of signs,

5) sample of nasal secretion taken via placement of a 7-mm diam. sponge on the septum for 30 sec, and

6) sample of tear fluid taken via placement of a 3-mm diam. sponge at the lower lid for 2-min,

On Monday through Thursday, the following will be performed before the subject enters the chamber:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination of the nose, eyes, and throat, and rating of signs,

4) sample of nasal secretion taken,

5) sample of tear fluid will be taken,

6) office spirometry to establish forced vital capacity (FVC) and forced expiratory volume at 1 sec (FEV_1),

7) exhaled nitric oxide (NO) measured via the mouth while exhaling through an expiratory resistance to indicate NO generated in the lungs, and

8) nasal resistance measured by active, anterior rhinomanometry.

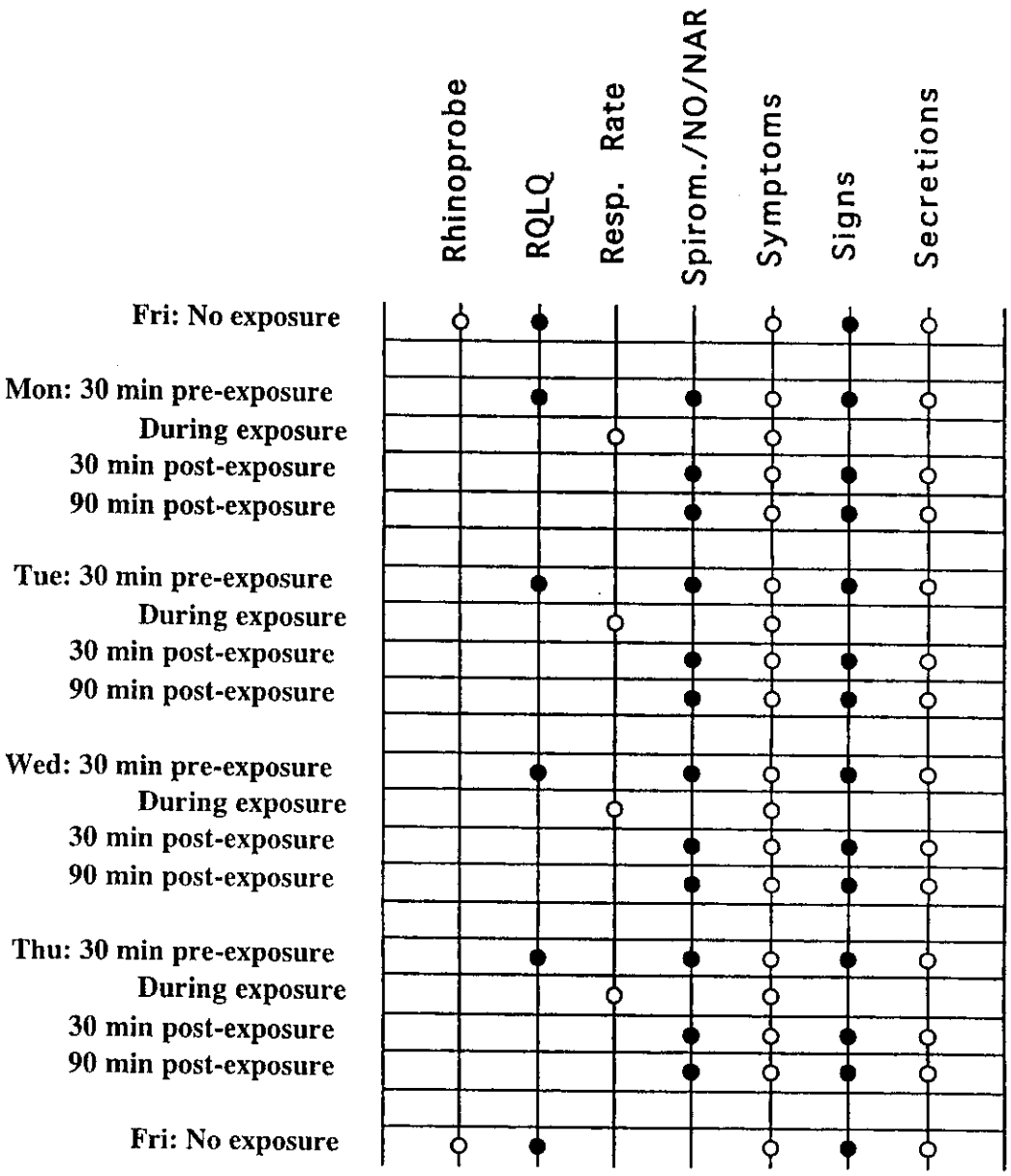
The following will then be performed in the chamber:

1) subject enters the chamber,

2) respiratory rate monitored remotely via Respibands placed around the thorax, and

3) in the last half-minute of exposure, the subject rates symptoms.

Table 3


Rhinoprobe
RQLQ
Resp. Rate
Spirom./NO/NAR
Symptoms
Signs
Secretions
Fri: No exposure
Mon: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Tue: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Wed: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Thu: 30 min pre-exposure
During exposure
30 min post-exposure
90 min post-exposure
Fri: No exposure

Thirty min and 90 min after a session, the following will be performed:

1) subject rates symptoms,

2) clinical examination, with rating of signs,

3) nasal secretion taken, as before the session ,

4) tear fluid taken, as before the session,

5) office spirometry, as before the session,

6) measurement of nasal resistance, as before the session, and

7) exhaled NO measured, as before the session.

On Tuesday, Wednesday, and Thursday, the cycle will be repeated, i.e., a pre-session evaluation and two post-session evaluations.

Approximately 24 hr after the Thursday session, the subject will return and the following performed:

1) subject fills out the RQLQ,

2) subject rates symptoms,

3) clinical examination, with ratings of signs,

4) nasal secretion collected, and

5) Rhinoprobe sample taken from the opposite nostril from the first.

Data Analysis: For interpretation of the study, we will distinguish between variables meant to monitor safety and those of substantive interest.

With respect to safety:

Respiratory rate will be monitored to examine whether the subject remains relaxed during exposures. An unexpected rise can indicate anxiety and gives reason to query the subject about any perceived threat.

Results of the office spirometry will be compared before and after exposure to examine whether a subject has experienced bronchoconstriction from the exposure. A reduction of FEV_1 of 15 % will be reason to remove a subject from further exposures at the level that caused the constriction.

Exhaled nitric oxide (NO) will be compared between after and before to examine whether a subject has developed any inflammation in the lungs.

With respect to substantive interest:

Score on the RQLQ will be analyzed for effects of level of exposure (0 ppm, 0.1 ppm, 0.15 ppm), of day as an indicator of total exposure, and of sex.

Ratings of symptoms (scores per site and total score) will be analyzed for effects of level of exposure, of time since exposure (30 min and 90 min post-exposure), of day as an indicator of total exposure, and of sex.

Ratings of signs on the clinical examination will be analyzed similarly to the ratings of symptoms. Ratings of symptoms and signs will be compared for associations between them.

The secretions from both the nose and the eye will be assessed for total mass collected and for the markers albumin, IL-8, and sICAM. The quantities of the biochemical indices will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex. The quantities will also be compared for associations with ratings of symptoms, ratings of signs, and the RQLQ.

Nasal resistance will be compared before vs. after exposure as an aspect of dose of chloropicrin vapor. A blocked nasal passage means lower dose than a patent passage. An increase in resistance is a normal response to inhalation of an irritating vapor. Nasal resistance will be analyzed for effects of level of exposure, of time since exposure, of day as an indicator of total exposure, and of sex.

The cells in the Rhinoprobe samples will be compared from the first to the sixth days as an index of cumulative effect of exposure.

7. Human Subjects

Inclusion Criteria for Normal Subjects:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

4) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Normal Subjects:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses.

8) Abuse of alcohol (more than three drinks of alcohol a day over the last year).

9) Use of mood altering drugs within the last year.

10) Use of tobacco or smoking of any substance including marijuana within the at year.

11) Daily use of medication, excluding birth control pills, but including nose drops or sprays, such as Afrin.

12) Impaired sense of smell.

13) Pregnancy at the time of the study.

14) Evidence of active infection, rhinitis, pharyngitis, clinically-significant inflammation in the nose or throat, or certain structural abnormalities in these regions.

15) Evidence of conjunctivitis, abnormal redness of the eyes, or abnormalities of the surface of the eyes.

16) Clinically elevated nasal resistance.

17) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of any of these conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

Sense of Smell: Screening for smell will entail taking a standardized test, e.g., the Connecticut Chemosensory Clinical Research Center [CCCRC] Test for odor threshold and odor identification (Cain, 1989). Subjects will excluded if their olfactory ability falls below normal by the criteria of the test.

Nasal Resistance: The assessment of nasal resistance will occur both in screening and in testing in Phase 3b. Nasal resistance will be measured via a system of computerized anterior rhinomanometry (RHINO; MultiSpiro, San Clemente, CA) that avoids deformation of the nares. The system relies upon an oxygen-type face-mask to monitor flow from one nostril while a tube sealed via a pressure patch (Rhino Diagnostics, Inc., San Diego, CA) monitors pressure at the other nostril.

During testing, the subject breathes normally through the mask through four cycles per nostril. Signals for pressure and flow are digitized and used to calculate resistance at –1.5 cm water column. Subjects with clinically abnormal resistance, defined as >5 cm H_20/ L/sec will be excluded in screening.

Nasal Cytology: Nasal cytology will be used both in screening and in testing in Phase 3b. Subjects who pass the clinical screening examination of the nose, throat, and eyes may still show evidence of inflammation in cytological analysis. In order to obtain cells, the investigator will use a Rhinoprobe, a flexible curette with a 1-mm diam. cup. Sampling with the Rhinoprobe entails a gentle scrape of 3-mm length along the superficial nasal mucosa of the lower turbinate under visual inspection. The procedure causes minor discomfort for an instant.

For analysis, the specimen is gently spread over a small area of a microscope slide, fixed in 95% alcohol, and stained with Wright-Giemsa stain. It is examined under low power (100 x) to determine the adequacy of the specimen and the areas of interest and then graded under high power (1000 x) for cells.

Nasal cytology can reveal various conditions, as follows (see Table 4): 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection, 3) large numbers of eosinophils or basophilic cells (3+ or 4+) provide evidence of inflammation. When used as an outcome variable in testing in Phase 3b, cells will be counted exactly.

Spirometry: Spirometry will be used both in screening and in testing in Phase 3b. Office spirometry testing will conform to the recommendations of the American Thoracic Society. Data to be acquired from this test include the following: FVC, the total volume of gas exhaled after a full inspiration, and FEV_1, the gas volume exhaled in one second by a forced expiration from a full inspiration. The data will allow calculation of the ratio FEV_1/FVC. Subjects whose pulmonary function fails to lie at or above 75% of predicted FEV_1 or FVC will be excluded.

Ocular Cytology: Ocular cytology will be performed in screening on impression-samples taken from the conjunctival membrane inside the lower eyelid. The lower lid

will be sampled with a 3-mm diam. membrane filter placed at the end of a rod. The rod weighs 60 g and suspension of the rod within a short outer cylinder at the moment of contact insures that 60 g will be exerted on the lid. The samples will be analyzed for the presence of cells in the same way as in nasal cytology.

Table 4
Guide for Grading Nasal Cytograms

Quantitative Analysis	Semi-quantitative Analysis	Grade
Epithelial Cells		
N/A	Normal morphology	N
N/A	Abnormal morphology	A
N/A	Ciliocytophthoria	CCP
Eosinophils, neutrophils		
0*	None	0
0.1-1.0*	Occasional cells	0.5+
1.1-5.0*	Few scattered cells or small clumps	1+
5.1-15.0*	Moderate number of cells and larger clumps	2+
15.1-20.0*	Larger clumps of cells which do not cover the entire field	3+
>20*	Large clumps of cells covering the entire field	4+
Basophilic cells		
0*	None	0
0.1-0.3*	Occasional cells	0.5+
0.4-1.0*	Few scattered cells	1+
1.1-3.0*	Moderate number of cells	2+
3.1-6.0*	Many cells easily seen	3+
>6.0*	Large number of cells, as many as 25 per high power field	4+
Bacteria**		
N/A	None seen	0
N/A	Occasional clump	1+
N/A	Moderate number	2+
N/A	Many easily seen	3+
N/A	Large numbers covering the entire field	4+
Goblet cells***		
0	None	0
1-24%	Occasional to few cells	1+
25-49%	Moderate number	2+
50-74%	Many easily seen	3+
75-100%	Large number, may cover entire field	4+

* Mean of cells per 10 high power fields (x1000)
** Note presence of intracellular bacteria
*** Ratio of goblet cells to epithelial cells, expressed as percentage

Persons with significantly elevated levels of PMNs (neutrophils, eosinophils, and basophilic cells) in the conjunctival membrane will be excluded. Persons with small elevations above normal will not.

Pregnancy: Testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded.

Inclusion Criteria for Subjects with Allergic Rhinitis:

1) Subjects must demonstrate their willingness to participate in the study and comply with its procedures by signing a written consent form.

2) Subjects must be 18-35 years of age, of either sex and any race.

3) Subjects must show evidence of allergic rhinitis.

4) Subjects must be free of any clinically significant disease that would interfere with evaluations in the study.

5) Female subjects must be willing to take a periodic test for pregnancy.

Exclusion Criteria for Subjects with Allergic Rhinitis:

1) History of occupational exposure to chloropicrin.

2) History of chronic cough, chronic pulmonary disease, chronic sinusitis, or nasal polyposis.

3) History of acute or chronic cardiovascular, liver, or kidney disease.

4) Acute illness within the previous month.

5) Investigational exposure to pollutants within one week.

6) History of chemical hypersensitivity.

7) History of ocular abnormalities, other than a need for glasses.

7) History of ocular abnormalities, other than a need for glasses.

8) Abuse of alcohol (more than three drinks of alcohol a day over the last year).

9) Use of mood altering drugs within the last year.

10) Use of tobacco or smoking of any substance including marijuana within the at year.

11) Daily use of medication, excluding birth control pills.

12) Absent sense of smell.

13) Evidence of active infection or certain structural abnormalities in the upper airways.

14) Pregnancy at the time of the study.

15) Impaired pulmonary functioning.

In an initial visit to the laboratory, subjects will give a medical history and, if not excluded by history, go through various screening tests, as indicated below:

Exam of the Nose, Throat, and Eyes: Examination of the nose and throat will entail direct visualization under head-lamp illumination for signs of rhinitis, pharyngitis, other inflammation in the throat or upper airways, or significant structural abnormalities, such as a markedly deviated septum. The examination of the eyes will entail inspection under slit-lamp illumination for conjunctivitis or other inflammation (e.g., swelling of the lids), ocular surface abnormalities (scarring, ulceration), and abnormal redness. The presence of signs of allergic rhinitis should be present in the patients. Signs of other conditions at a degree greater than 1 on the 0-3 rating of signs will constitute grounds for exclusion (see Table 2 for interpretation of scale).

As part of the examination, the examiner will also establish if there is a history of seasonal or perennial allergic rhinitis with positive skin test to one or more allergens within previous 12 mo. If skin test results are unavailable, a test will be performed at the Nasal Dysfunction Clinic under the supervision of Dr. Terence Davidson. Positive evidence will be a criterion for inclusion. The examiner will also ask about symptoms. A score ≥ 8 for the combined nasal and eye symptoms, with congestion score ≥ 2 for at least three of the five days prior to screening will be a criterion for inclusion.

Sense of Smell: Screening for smell will entail the same testing as described for normals. Subjects will excluded if their olfactory ability falls into the anosmic zone, i.e., if the subject evinces no olfactory ability whatsoever.

Nasal Resistance: The assessment of nasal resistance will entail the same testing as described above. Subjects will be excluded if their resistance lies above 6 H_2O/L/sec.

Nasal Cytology: Nasal cytology will be performed as described above. Subjects will be excluded if they show the following in their cytograms: 1) the presence of ciliocytophthoria (clumping of chromatin) in epithelial cells provides evidence for respiratory viral infection, or 2) the presence of large numbers of neutrophils (3+ or 4+) with intracellular bacteria provides evidence of bacterial infection.

Spirometry: Screening will entail the same methods as described for normals.

Ocular Cytology: Screening will be performed by the methods described above for normals. Subjects will not be excluded by this test, except for signs of infection, but the results will be compared with those of the nasal cytology for possible stratification of the sample of subjects by presence or absence of inflammatory cells in the eye.

Pregnancy: Testing of female subjects for pregnancy (urine testing) will be conducted as part of the initial screen and weekly throughout the study. Pregnant women will be excluded.

8. Informed Consent

Subjects will be recruited from the general population. Formal consent will be sought just prior to screening, typically by staff involved in the screening tests. The consent form will contain the relevant facts regarding the reason for the study, the task at hand, any possible adverse effects, and the opportunity to withdraw at any time without penalty. Consent will be written and the subject will receive a copy of the form along with the subject's bill of rights.

9. Therapeutic Alternatives

Not a therapeutic study.

10. Potential Risks

Screening: The screening procedures of direct visualization of the nasal and ocular mucosae and measurement of nasal resistance pose no realistic risks that we can anticipate. Sampling from the nasal mucosa has been performed routinely by Dr. Jalowayski in the Nasal Dysfunction Clinic for many years and entails minimal risk. The impression cytological sampling from the lower lid, also entails minimal risk.

Testing: The risks associated with testing entail sensory irritation of the upper airways or eyes and remotely in these circumstances asthma. We say "remotely" because the exposures will be to perithreshold concentrations with respect to irritation.

11. Risk Management

Monitoring: Subjects will be monitored visually during testing. Testing will stop if the subject finds the test situation uncomfortable and asks to stop. We will instruct subjects to report any respiratory symptoms immediately.

Actions to be taken if an adverse event occurs:

Upper Airway or Ocular Discomfort: Irritation of the nose or eyes should subside soon after exposure. Should effects last longer than expected, the subject will be instructed regarding how to irrigate the affected area with saline solution. An eye irrigation system exists within the laboratory. Furthermore, over-the-counter preparations for such purposes will be available on-site for the subject to use before leaving the lab and to take home. The subject will be informed that if any such irritation or discomfort fails to show progressive improvement during the ensuing hours, then the subject should page Dr. Terence Davidson who will oversee medical management of the symptoms.

Lower airway reactions: Should exposure cause shortness of breath from

apparent bronchoconstriction, then an ACLS-certified staff member will initiate an asthma-management protocol. An emergency kit to treat acute asthma will include: a) a Ventolin inhaler (albuterol USP inhalation aerosol), b) an AeroChamber to be used with the metered dose inhaler, c) a nebulizer and oxygen source, d) a pulse oxymeter, and e) an epinephrine auto-injector (EpiPen) to deliver a 0.3 mg intramuscular dose of epinephrine (1:1000), and d) apparatus for oxygen therapy.

The protocol for management of acute asthma will be the following: a) if symptoms occur, the subject will be offered treatment with Ventolin (one puff held in the lungs for 10 sec., followed by a second puff 30 sec later), b) the pulse oxymeter will be attached to a digit in order to monitor oxygen saturation, c) if the subject seems unable to inhale from the inhaler adequately, then the nebulizer will be used, d) Dr. Thomas Bruff will be paged and informed of the event, and he will decide how the subject should be managed, e) if the subject is not in acute respiratory distress, he will be asked to remain in the lab for the next hour; the subject will be given Dr. Bruff's beeper number when released;; Dr. Bruff will contact the patient within 2-4 hours and will arrange any further care, including office follow-up, if necessary f) if the subject fails to obtain relief, he will be transported to the emergency room at Thornton Hospital, less than 2 miles from the lab; management of the subject in the ER will depend upon their standard protocols; and g) if the subject shows signs of anaphylactic shock while at the laboratory, he can be given an injection of epinephrine from the EpiPen and administered humidified oxygen; in that case, 911 will be called.

12. Potential Benefits

There will be no benefits to individual subjects.

This research is motivated by the need to develop a definitive set of data relevant to environmental and occupational exposures to chloropicrin. Since the chemical has very low systemic toxicity compared to its rather strong irritating properties, it is irritation that will dictate permissible exposure levels. (The irritation response, as we have noted, largely protects against systemic exposure.) In so far as the present research makes it possible to set more scientifically defensible exposures, then its potential benefit to occupational and public health is considerable.

Approximately 50% of the ACGIH TLV's are based on sensory irritation and yet there have been almost no controlled studies of such. Companies and trade groups that exercise product stewardship over commodity chemicals seek quantitative data on human sensory irritation. As product stewards, these entities are often asked to advise users of their products about hazardous properties. Indeed, they must do so in Material Safety Data Sheets. This matter goes beyond setting permissible exposure levels into more intrinsic understanding of the perceptual and physiological effects of the chemicals. We see these developments as salutary.

13. Risk-Benefit Ratio: Minor risk, no individual benefits, but benefits to society as indicated just above.

14. Expense to subject: None anticipated.

15. Payment for Participation: Subjects will receive $15 per hour for participation. subjects can receive their payment (cash) at the end of a session or can allow it to accumulate over sessions by mutual agreement. Subjects will not be reimbursed for travel.

16. Privileges/Certifications and Licenses: Dr. Davidson has privileges at UCSD Medical Center. He indicated to the Human Subjects Program in 1999 that sampling from mucosal tissue, the only procedure where we touch the subject, does not require direct medical supervision. Dr. Jalowayski, who has worked with Dr. Davidson in the Nasal Dysfunction Clinic for more than 15 years, developed the sampling and performs it routinely in the clinic. Dr. Jalowayski will do the sampling in this project.

Dr. Thomas Bruff, board certified in internal medicine and in occupational medicine, is attending staff at UCSD Medical Center. He is ACLS-certified.

17. Bibliography:

American Conference of Governmental Industrial Hygienists (1991). Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th ed. Cincinnati: ACGIH.

Cain, W. S. (1989). Testing olfaction in a clinical setting. Ear, Nose, and Throat Journal, 68, 316-328.

Calderon, M. A, Devalia, J. L, Prior, A. J, Sapsford, R. J, & Davies, R. (1997). A comparison of cytokine release from epithelial cells cultured from biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis..Journal of Allergy and Clinical Immunology, 99, 65-76.

Ciprandi, G., Buscaglia, S., Pesce, G., Villaggio, B., Bagnasco, M., & Canonica, G.W. (1993). Allergic subjects express intercellular adhesion molecule – 1(ICAM-1 or CD54 on epithelial cells of conjunctiva after allergen challenge. Journal of Allergy and Clinical Immunology, 91(2), 783-792.

Ciprandi, G, Pronzato, C, Passalacqua, G, Ricca, V, Bagnasco, M, Grogen, J, Mela, G. S. & Canonica, G. W. (1996). Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells: An antiallergic activity. Journal of Allergy Clinical Immunology, 98(6), 1088-96.

Granstrand, P., Nylander-French, L., & Holmstreom, M. (1998). Biomarkers of nasal inflammation in wood-surface coating industry worker. American Journal of Industrial Medicine, 33(4), 392-9.

Jalowayski, A. A. & Zeiger, R.S. (1988). Examination of nasal or conjunctival epithelium specimens. In Manual of Allergy and Immunology, eds. Lawlor, G. J. and Fischer, T.J.; Little, Brown and Co. Boston/Toronto. Pp. 432-434.

Juniper, E. F., Thompson, A. K., Ferrie, P. J., & Roberts, J. N. (2000). Development and validation of the Mini Rhinoconjunctivitis Quality of Life Questionnaire. Clinical and Experimental Allergy, 30, 132-140.

Klimek, L, Wolf, H, Mewes, T, Dormann, D, Reske-Kunz, A, Schnitker, J & Mann, W. (1999). The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophilic cationic protein and tryptase in nasal secretions. Journal of Allergy and Clinical Immunology, 103, 47-53.

Kobayashi, R. H., Beaucher, W. N., Koepke, J. W., Luskin, A. et al. (1995). Triamcinolone acetonide aqueous nasal spray for the treatment of patients with perennial allergic rhinitis: A multicenter, randomized, double-blind, pacebo-controlled study. Clinical Therapeutics, 17, 503-513.

Krieger, R. I. (1996). An assessment of implied worker exposure and risk associated with chloropicrin loading, application, and field tarping activities following application, and implied exposure and risk for off-field concentrations resulting from soil fumigation. Registration document submitted to CAL-EPA Department of Pesticide Regulation, October 1996.

Meltzer. E.O., Jalowayski, A.A., Orgel, H.A., & Harris, A. G. (1998). Subjective and objective assessments in patients with seasonal allergic rhinitis: Effects of therapy with mometasone furoate nasal spray. Journal of Allergy and Clinical Immunology, 102 (1), 39-49.

Polito, A. J. & Proud, D. (1998). Epithelial cells as regulators of airway inflammation. Journal of Allergy and Clinical Immunology, 102(5), 714-718.

Silkoff, P. E., Robbins, R. A., Gaston, B., Lundberg, J. O. N., & Townley, R. G. (2000). Endogenous nitric oxide in allergic airway disease. Journal of Allergy and Clinical Immunology, 105 (3), 438-448.

U. S. Environmental Protection Agency (1984). Notice to Manufacturers, Formulators, Producers and Registrants of Fumigant Products. PR Notice-84-5.

Van Cauwenberge, P., Juniper, E. F., & the STAR Investigating Group (2000) Comparison of the efficacy, safety and quality of life provided by fexofenadine hydrochloride 120 mg, laratadine 10 mg and placebo administered once daily for the treatment of seasonal allergic rhinitis. Clinical and Experimental Allergy, 30, 891-899.

Wagenmann, M., Baroody, F. M., Desrosiers, M., Hubbard, W. S., Ford, S., Lichtenstein, L. M., & Naclerio, R. M. (1996). Unilateral nasal allergen challenge leads to bilateral release of prostaglandin D_2. Clinical and Experimental Allergy, 26, 371-378.

Wilson, S. J, Lau, L., & Howarth, P. H. (1998). Inflammatory mediators in naturally occurring rhinitis. Clinical and Experimental Allergy, 28(2), 220-7.

18. Industry Studies: Supported by the Chloropicrin Manufacturer's Task Force. The Task Force approached the Chemosensory Perception Laboratory about the need for the information and we designed the study. The UCSD Office of Contracts and Grants Administration negotiated a contract with the Task Force. The University will retain all patent rights to the results and is free to publish the work. The PI has no personal agreements (e.g., consultation, confidentiality) with the Chloropicrin Manufacturer's Task Force.

19. Other Funding: None.

20. Cancer Studies: Not applicable.

21. Biological Materials Transfer Agreement: No materials to be transported.

22. Investigational Drug Fact Sheet: No investigational drugs to be used.

23. Conflict of Interest: Form submitted here.

24. Nursing Staff: No impact.

25. Information Service: Will be completed.

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to WS3 (Phase 3a)

William S. Cain, Ph.D. and associates from the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of feel and odor from a chemical called WS3. The chemical is used commonly to impart some sense of feel in personal products. The results are intended to provide information regarding certain testing procedures we will use in later studies on chemical feel.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately 12 sessions of up to 2.5 hours over a period of a 2-3 weeks.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to WS3, you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by-Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to make judgments about the presence of feel from WS3, to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests.

You will receive $15 per hour for your time in screening and in testing. If you compete all testing, we estimate that you will earn an amount in the vicinity of $450.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-

investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (619-407-1606).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late or fail to show up for scheduled sessions.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 455-5050 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor (Phase 3b)

William S. Cain, Ph.D., and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, in subsequent sessions we will perform various tests of how your nose and eyes respond to one-hour exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in three six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all 6 days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15 per hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $700. There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and

painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to chloropicrin, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (619-407-1606).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late, fail to show up for scheduled sessions, and cancel sessions repeatedly or at the last minute, or if we feel that you cannot perform the judgments within normal bounds of competence.

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call (858) 455-5050 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

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By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to WS3 (Phase 3a)

William S. Cain, Ph.D. and associates from the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of feel and odor from a chemical called WS3. The chemical is used commonly to impart some sense of feel in personal products. The results are intended to provide information regarding certain testing procedures we will use in later studies on chemical feel.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately 12 sessions of up to 2.5 hours over a period of a 2-3 weeks.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to WS3, you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by-Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to make judgments about the presence of feel from WS3, to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests.

You will receive $15 per hour for your time in screening and in testing. If you compete all testing, we estimate that you will earn an amount in the vicinity of $450.

There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for public health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-

investigators. If such a reaction should occur after you have left, we will have you page Dr. Bruff directly (619-407-1606).

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You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor (Phase 3b)

William S. Cain, Ph.D., and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. In addition, for women, a pregnancy test on a urine sample will be required. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one. If you don't pass screening, you will be excused and paid for your time.

After the screening session, in subsequent sessions we will perform various tests of how your nose and eyes respond to one-hour exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in three six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all 6 days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15 per hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $700. There will be no direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for health regarding exposure to chloropicrin.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and

painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

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_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You agree to participate in the research described above. You may keep a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights."

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date

To: File
From: W. S. Cain
Re: Report on symptoms of [redacted]
Date: 13 November 2002

On Friday May 24th, I read a message (exhibit A) sent to my assistant Kevin Magruder on May 23rd in which [redacted] had said that he could not come in for testing on the 24th because he had become sick and that the testing with chloropicrin might have been partially responsible. I had been out of town visiting US Borax in Valencia, CA and out of touch with e-mail on the 23rd.

I had been present the day of [redacted] exposure to chloropicrin the previous Friday (May 17th). This had been his first and only exposure to chloropicrin. Our only previous contact with him had come the previous Friday (May 10th), the day of his successful screening

At approximately 4:45 PM on of his day of testing with chloropicrin, [redacted] complained of feeling a bit light-headed. This was brought to my attention, and he and I spoke about his symptom. He said it was not serious, but that he wanted to mention it. We sat in the fresh air for a few minutes and I asked him if he wished to discontinue the test. I made it clear that he would be paid for the entire day even if he chose to discontinue. He knew that he had about 20 minutes more work (eight exposures plus a 5-minute break) and said that he would be happy to finish. I stayed just outside the testing facility until the testing ended just after 5 PM. At that time, [redacted] said that he no longer felt light-headed, so I allowed him to leave.

In the following week, [redacted] and Kevin arranged for [redacted] next appointment, May 24th. On the morning of the 24th, after reading his e-mail, I asked him by e-mail what his symptoms were (exhibit B). He responded on the weekend, which happened to be Memorial Day weekend, and related his symptoms. When I read his message, I asked him to give me a phone number so that we could discuss the matter (exhibit C).

In his message to me (exhibit C) and in our conversation on Monday, the 27th [redacted] said that his symptoms had begun the day before the testing. He told me that his roommate and various friends had been ill with upper respiratory infections during that week and that he had assumed he was coming down with the same illness. When he came for testing on the 17th, he had the symptoms of a cold/flu. He did not tell us that. He did indicate on a rating of symptoms before testing mild nasal congestion, cough, hoarseness, dryness, and irritation (Exhibit D). At that time, the assistant did not bring this pattern of ratings to the attention of Dr. Jalowayski who was at the lab at the beginning of the testing.

[redacted] explained to me on the 27th that he had gone to the student health services on the 24th for a doctor's opinion. The doctor told him that he had a viral infection and she gave him some medication for his nasal symptoms. [redacted] also mentioned that he was long prone to nosebleeds, particularly when he had a cold. He had not told this to the doctor, who might not have given him the medication if she had known that. I told him that I would call him during the week to see whether he was improving. I called him the

next day, the 28th. He said that he was feeling a little better. In subsequent conversations during that week, he related that he felt incrementally better, but still not well. He went back to student health services Friday, the 31st and, as I recall, was given some cough medicine. On that day, I also sent a message to one of [redacted] teaching assistants regarding [redacted] bout of illness over the previous days (exhibit E). [redacted] had missed some homework assignments and did not have a treating physician's note to explain his illness.

I called [redacted] at the beginning of the following week and he was progressing out of his illness. We discussed the possibility, as we had earlier, that I might arrange to bring him to Dr. Davidson, an ENT specialist. [redacted] expressed some interest in this, but then his symptoms were resolving well. Our last contact took place on approximately June 4th.

My impressions: I was convinced from his message to me and from our subsequent conversation that the testing had not caused [redacted] symptoms. On the day of testing, [redacted] had been asked to detect the odor of chloropicrin over a total of 120 trials. He was exposed to four concentrations ranging from 356 to 1,200 ppb. He did not detect odor perfectly at any of these concentrations, but he showed a normal progression of improving detection from the lowest to the highest concentration. His performance was quite typical. There were no mishaps that led to over-exposure. The gas-chromatographic readings of the concentrations on the day were within normal limits.

As I said to [redacted] teaching assistant, the question of whether the testing contributed to [redacted] symptoms will probably never be known. No matter what, I was concerned about his health, as I would have been for any student who was suffering as he was. (It is perhaps relevant to note that as a member of the staff of the UCSD Nasal Dysfunction Clinic I am accustomed to interpreting patients' respiratory symptoms. Nothing about [redacted] symptoms struck me as out of the ordinary for a bad cold.)

[redacted] cold started toward the end of the school year when many assignments were due. Although I was agreeable to sending the message to his teaching assistant, the thought occurred to me that [redacted] might ask for notes for other missed work or even for not taking final exams, which were to begin on June 10th. I saw the possibility for some manipulation. As I sensed significant improvement, I decided to let [redacted] contact me if he needed further help or advice. I felt that he would not hesitate to do so. Although I had initiated all contacts beginning on the 24th, I sometimes had to leave a message for [redacted] to call me back. He did so in all instances, so I felt that he would not hesitate to ring me. I did not hear from him after June 4th.

Regarding the acceptance of [redacted] ratings on the day of testing, we realized that the assistant had behaved according to instructions, but the instructions had not foreseen a pattern of ratings such as [redacted] Occasionally, subjects would give a rating of mild, a 1 on a scale of 0 to 3, for one or another symptom. We had instructed the assistant to bring ratings of 2 or higher to the attention of the doctor on site. This would have triggered a discussion with the subject regarding the nature of the symptoms. We had not anticipated a series of 1's such as [redacted] gave. This has been corrected.

Sent 5/23/02

```
Content-Type: text/plain;
        charset="iso-8859-1"
Content-Transfer-Encoding: quoted-printable

Kevin,
   I do not think I can come in tomorrow for testing. Over the past =
week, I have become very sick. I have a feeling that the testing may be =
partially responsible.

[redacted]

------=_NextPart_000_000C_01C20269.F812D2C0
Content-Type: text/html;
        charset="iso-8859-1"
Content-Transfer-Encoding: quoted-printable

<!DOCTYPE HTML PUBLIC "-//W3C//DTD HTML 4.0 Transitional//EN">
<HTML><HEAD>
<META http-equiv=3DContent-Type content=3D"text/html; =
charset=3Diso-8859-1">
<META content=3D"MSHTML 6.00.2713.1100" name=3DGENERATOR>
<STYLE></STYLE>
</HEAD>
<BODY bgColor=3D#ffffff>
<DIV><FONT face=3DArial size=3D2>Kevin,</FONT></DIV>
<DIV><FONT face=3DArial size=3D2>    I do not think I can =
come in=20
tomorrow for testing. Over the past week, I have become very sick. =
I have a=20
feeling that the testing may be partially responsible.</FONT></DIV>
<DIV><FONT face=3DArial size=3D2></FONT> </DIV>
<DIV><FONT face=3DArial size=3D2>Trevor</FONT></DIV></BODY></HTML>

------=_NextPart_000_000C_01C20269.F812D2C0--

--0-735774545-1022196570=:61393--
```

Exhibit B

Subject: Cancel
Date: Fri, 24 May 2002 08:19:42 -0700
From: "William S. Cain" <wcain@ucsd.edu>
To: [redacted]

[redacted] I have just seen your e-mail. Please tell me what symptoms you have had this week and how you think your exposure in our lab may have contributed to them. -Dr. Cain

--
William S. Cain, Ph.D.
Chemosensory Perception Laboratory
Dept. of Surgery (Otolaryngology)
University of California, San Diego
La Jolla, CA 92093-0957
Phone: +858-622-5831
Fax: +858-458-9417
e-mail: wcain@ucsd.edu

http://www.ucsd.edu/chemo

Exhibit C

Subject: Re: Cancel
Date: Mon, 27 May 2002 16:37:22 -0700
From: "William S. Cain" <wcain@ucsd.edu>
[redacted]

[redacted] Please give me a phone number so that I might speak to you. -Dr. Cain

[redacted] wrote:

> Dr. Cain,
> I have had had the following symptoms, starting from about Thursday, May
> 16th. Throat irritataion, persistent coughing, fever and headache,
> dizziness, nasal congestion, back pains, nausea, bloody noses, and
> difficulty breathing.
> The exposure to the lab i think may have contributed to my difficulties
> breathing, which leads to the heavy and frequent coughing. These have also
> been the longest lasting symptoms, with not much improvement either.
>
> Regards,
> [redacted]
> ----- Original Message -----
> From: "William S. Cain" <wcain@ucsd.edu>
> To: [redacted]
> Sent: Friday, May 24, 2002 8:19 AM
> Subject: Cancel
>
> [redacted] I have just seen your e-mail. Please tell me what symptoms you
> > have had this week and how you think your exposure in our lab may have
> > contributed to them. -Dr. Cain
> >
> > --
> > William S. Cain, Ph.D.
> > Chemosensory Perception Laboratory
> > Dept. of Surgery (Otolaryngology)
> > University of California, San Diego
> > La Jolla, CA 92093-0957
> > Phone: +858-622-5831
> > Fax: +858-458-9417
> > e-mail: wcain@ucsd.edu
> >
> > http://www.ucsd.edu/chemo
> >
> >

--
William S. Cain, Ph.D.
Chemosensory Perception Laboratory
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T

Ex. 27

Rating of Symptoms

SID: PICM43

Date: 5/17/02 Examiner: KM
Time: 9:45am

Use the following scale to indicate the degree of symptoms:

Scale	Degree	Meaning
0	None	No symptom.
1	Mild	Symptom present, but minimal awareness, easily tolerated.
2	Moderate	Symptom definite and bothersome, but tolerated.
3	Severe	Symptom hard to tolerate and can interfere with activities of daily living or sleeping.

Nose			
a.	Congestion	1	
b.	Runny Nose	0	
c.	Itchiness/Sneezing	0	
d.	Irritation	0	1
Eye			
a.	Tearing	0	
b.	Puffiness	0	
c.	Itchiness	0	
d.	Irritation	0	0
Throat			
a.	Cough	1	
b.	Hoarseness	1	
c.	Dryness	1	
d.	Irritation	1	4
		Total Score:	5

Examiner's signature: _____

Exhibit E

From - Wed Nov 13 21:26:02 2002
X-Mozilla-Status: 0001
X-Mozilla-Status2: 00000000
Message-ID: <3CF7C9BB.8704503F@ucsd.edu>
Date: Fri, 31 May 2002 12:06:40 -0700
From: "William S. Cain" <wcain@ucsd.edu>
X-Mailer: Mozilla 4.75C-CCK-MCD {C-UDP; EBM-APPLE} (Macintosh; U; PPC)
X-Accept-Language: en,pdf
MIME-Version: 1.0
To: [redacted]
CC: [redacted]
Subject: [redacted]
Content-Type: text/plain; charset=us-ascii; x-mac-type="54455854"; x-mac-creator="4D4F5353"
Content-Transfer-Encoding: 7bit

Dear [redacted]

A couple of weeks ago (5/17), [redacted] served all day in some perceptual testing of the odor of a vapor that can irritate as well as evoke odor. [redacted] had been screened for healthy airways (absence of infection and allergic reactions) in a day before his participation. On the day before the testing (5/16), [redacted] had begun to feel symptoms of an impending infection. He indicated on a form on 5/17 that he had minor symptoms, which would not in and of themselves have disqualified him from participation. We would, however, have disqualified him if we knew that the symptoms were progressive and indeed they did progress. In the week after the testing, [redacted] was quite ill with symptoms of an upper and lower airway infection and flu. I was made aware of this toward the end of the week when [redacted] inquired about whether our testing had contributed to the severity of his symptoms.

[redacted] went to student health a week ago today and apparently was seen by a physician just at the very end of the day so that there was little contact time between physician and patient. I know that he left with some medication for his nasal symptoms. Over the last week I have sought to track [redacted] symptoms by phone and e-mail contact. He has shown improvement, though not evenly for all his symptoms. In light of his slow progress, I am hoping to have him seen by one of my colleagues in otolaryngology, perhaps today.

Last evening, [redacted] asked me to relate this situation to you. I can attest that [redacted] has been quite ill. The question of whether our testing contributed in any way to the severity of his illness will probably never be known. We all know from personal experience that one viral infection can hit us much harder than others and that there is no cure but time. This one hit [redacted] very hard. The propinquity between his symptoms and our testing has made me feel a responsibility to stay in touch with his condition.

-W. S. Cain, Professor of Surgery

--
William S. Cain, Ph.D.
Chemosensory Perception Laboratory
Dept. of Surgery (Otolaryngology)
University of California, San Diego
La Jolla, CA 92093-0957
Phone: +858-622-5831
Fax: +858-458-9417
e-mail: wcain@ucsd.edu

http://www.ucsd.edu/chemo

This page removed due to proprietary confidential information.

030246

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN RESEARCH PROTECTIONS PROGRAM

TO: Dr. William Cain Mailcode: 0957

RE: Project #030246
Human Sensory Irritation Testing for Chloropicrin

Dear Dr. Cain:

The above-referenced project was reviewed and approved by one of this institution's Institutional Review Boards in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56), including its relevant Subparts. This approval, based on the degree of risk, is for 365 days from the date of **IRB review and approval** unless otherwise stated in this letter. The regulations require that continuing review be conducted on or before the 1-year anniversary date of the IRB approval, even though the research activity may not begin until some time after the IRB has given approval.

Date of IRB review and approval: 3/4/2004

D. Masys

/cc

Daniel Masys, M.D., Director
Human Research Protections Program
Mailcode 0052 Phone: 858-455-5050
E-mail: hrpp@ucsd.edu

Note: All Human Subject research conducted at the VA facility and/or utilizing VA/VMRF funds MUST BE APPROVED by the VA Research and Development Committee prior to commencing any research.

Approval release date: 3/6/2004

IRB PROTOCOL MONITORING FORM

FIRST NOTICE

Project #: 030246

Date: January 13, 2004 YOUR RESPONSE IS DUE NOT LATER THAN: 2/19/2004
To: Dr. William Cain Mailcode: 0957
From: HUMAN RESEARCH PROTECTIONS PROGRAM (HRPP)
Re: Human Sensory Irritation Testing for Chloropicrin

FEB 17 2004

The DHHS, the FDA and the University of California REQUIRE that the IRB conduct continuing review of ongoing research at intervals appropriate to the degree of risk, but not less than once per year. This form constitutes part of this requirement. Please fill out the following IRB protocol monitoring form **based on subjects studied at this institution except as indicated in #2 and #3.**

1. Has the project been activated? Yes____ No✓ If yes, is it active now? Yes__ No ____

2. How many subjects have been studied to date at UCSD sites? 0 at all sites? ___

3. What is the expected accrual needed to complete the study at UCSD sites? 40 at all sites? ____

4. Have changes in the scientific literature or interim experience with this or related studies changed your assessment of potential risks or benefits to study subjects? No If yes, explain in Summary of Progress to Date below.

5. How many subjects enrolled on this protocol had: serious and unexpected reactions? 0 deaths unrelated to the protocol? 0 deaths possibly related? 0 deaths probably or definitely related? 0 withdrew before completing the project? 0 or complaints? 0 **Specify nature of complaint(s) in your summary. Include the date AEs were reported to the IRB. (If you have not yet notified the IRB of serious and unexpected, or unusual reactions or deaths, an explanation and completed UCSD Research Subject Injury Report must be filed immediately.)**

6. Is there a DSMB (Data Safety Monitoring Board) for this study? Yes___ No✓. If yes, have you forwarded all DSMB reports to the IRB? Yes____ No____.

7. Do you plan to make any changes in the project protocol? Yes_____ No✓

NOTE: ANY MODIFICATIONS IN THE PROTOCOL SHOULD NOT BE SUBMITTED WITH THIS ONE-YEAR MONITORING FORM.. AMENDMENTS MUST BE SUBMITTED AS SEPARATE ITEMS FOLLOWING THE GUIDANCE OUTLINED ON THE AMENDMENT FACT. AMENDMENTS MUST BE APPROVED BY THE IRB PRIOR TO INITIATION EXCEPT WHERE NECESSARY TO ELIMINATE APPARENT IMMEDIATE HAZARD TO THE STUDY PARTICIPANT. (ANY CHANGES IN ANTICIPATED RISKS OR BENEFITS THAT MAY OCCUR AS A RESULT OF CHANGES IN THE PROTOCOL MUST BE INCLUDED.)

8. **SUMMARY OF PROGRESS TO DATE. Please attach on a separate sheet,** a page that summarizes a) progress in conducting, monitoring, and analyzing the study; b) summary of serious and unexpected reactions; c) reason for any subject's, voluntary or involuntary, withdrawal from the study; d) preliminary results if available; e) changes in the scientific knowledge relevant to the conduct of this study; and f) discussion of adjustments in study design or consent forms that have been made during this period. g) If it is a multi-center trial, any information garnered from other centers that should be reported to the IRB should be included in the summary as well.

9. **A COPY OF THE CURRENT STAMPED APPROVED INFORMED CONSENT.**

Approval for this project will expire on **3/6/2004**. The latest date for receipt of this report to avoid expiration is **2/19/2004.** Failure to meet this deadline will require a complete application for this project. If you plan to continue this research, approval from the IRB is required. If there have been no changes in the protocol (or if changes have already been approved by the IRB), you may request continued approval in the space below.

Please indicate: RENEW: ✓ **DO NOT RENEW:**_____

WSCain 0957

Signature of Principal Investigator Mail Code

Date: 2/16/04 Phone Number 858-622-5831

RETURN 2 COLLATED SETS OF THE MONITORING FORM, SUMMARY, AND ONE COPY OF THE CURRENT STAMPED CONSENT FORMS TO: HUMAN RESEARCH PROTECTIONS PROGRAM, 0052. THIS RENEWAL CANNOT BE SUBMITTED ELECTRONICALLY.

To: HRRP
From: W. S. Cain, MC 0957
Re: Project #030246, Human Sensory Irritation Testing for Chloropicrin.

Date: 17 February 2004

8. Summary of Progress to Date:

   a) Progress in conducting, etc.: The study will begin in early March.

   b) Summary of experience: Not applicable.

   c) Reason for withdrawal: Not applicable.

   d) Preliminary results: Not applicable.

   e) Changes in scientific knowledge: Not Applicable.

   f) Discussion of adjustments: Not applicable.

   g) If a multi-center trial: Not applicable.

030246

University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to a Vapor Blend (Phase 3a)

William S. Cain, Ph.D. and associates from the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of a blend of menthol, camphor, and eucalyptus vapors. The vapor will come from a Sunbeam Waterless Vaporizer. The results are intended to provide information regarding certain testing procedures we will use in later studies on the perception of vapors.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in the study. You will be asked to participate in approximately seven sessions of up to 2.5 hours over a period of two weeks.

Procedures: If you agree to participate, the following will happen. Before you may serve in the main testing with the blend of vapors you will need to pass screening tests including a medical history and a brief test of your sense of smell. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. If you don't pass screening, you will be excused and paid for your time.

After the screening session, your task in subsequent sessions will be to rate how your nose, throat, and eyes feel, and to have exams of your nose and eyes, as in the screening tests. We will also take a sample of your nasal secretions by putting a little sponge on the wall between your nostrils and by putting some fluid into your nostrils and asking you to blow it out.

You will receive $15 per hour for your time in screening and in testing. If you complete all testing, we estimate that you will earn an amount in the vicinity of $275.

There will be no direct benefits to you of participation in this research. However, the investigators may learn about how to improve procedures to test biological effects of certain environmental agents not included in the present work.

We will advise you of any significant research findings relevant to your continued participation.

You will experience some discomfort from the testing, such as when we test your breathing, take a scraping from your nose, and take secretions. The discomfort should be mild and brief. We do not expect discomfort from the exposure to vapors. Nevertheless, you will be free to discontinue exposure at any instant. A staff member, who will always

be present, will guide you from the exposure. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, page Dr. Bruff directly at (619) 407-1606).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late or fail to show up for scheduled sessions.–

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call the Human Research Protections Program at (858) 455-5050 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators, the UCSD Institutional Review Board, and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

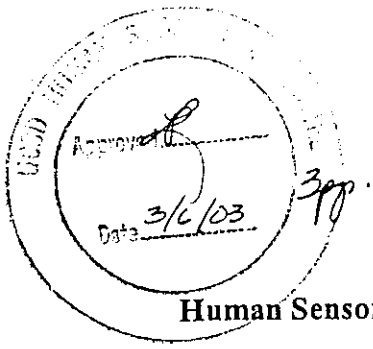
By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You will be given a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights" to keep.

You agree to participate.

_____ _____ _____

Subject's signature Witness Date

030246



University of California - San Diego

Consent to Act as a Research Subject

Human Sensory Reactions to Chloropicrin: Irritation and Odor (Phase 3b)

William S. Cain, Ph.D., and associates of the Chemosensory Perception Laboratory are conducting a research study, sponsored by the Chloropicrin Manufacturers Task Force, on the perception of irritation and odor from chloropicrin. The chemical is used commonly to fumigate fields for planting and as a warning agent in structural fumigation. The results are intended to provide information regarding safe levels of exposure for people who work with the material and for people who may be exposed to it unintentionally.

You are being asked to participate because you are between 18 and 35 years of age. There will be approximately 16 male and 16 female subjects in this phase. You will be asked to participate in approximately 20 sessions of up to 3 hours over a period of a few weeks and scheduled at mutual convenience.

Procedures: If you agree to participate, the following will happen. Before you will be exposed to chloropicrin, you will need to pass screening tests including a medical history and a brief test of your sense of smell to assure that you can smell everyday objects normally. The screening will also involve a) visual inspection of your nose and eyes, b) taking a small scraping of the lining from inside your nose, c) taking a little of the film from your lower eyelid with a blotting paper, and d) a test of breathing. These four tests (a-d), which are routinely performed in the clinic, will be administered by Alfredo Jalowayski, Ph.D. If you are a female and capable of child-bearing, a sample of urine will be collected before the study is begun in order to be as sure as possible that you are not pregnant. Your participation requires that you use a birth control method, such as abstinence, diaphragm, condom or intrauterine device to prevent pregnancy during the study, as chemicals inhaled at irritating levels could possibly harm an unborn child. If you miss a period or think you might be pregnant, you will notify the doctor. You may have to withdraw from the study. If you don't pass screening, you will be excused and paid for your time.

After the screening session, in subsequent sessions we will perform various tests of how your nose and eyes respond to one-hour exposures to low, but slightly irritating levels of chloropicrin vapor or to just air (control condition). We ask you to participate in three six-session blocks over a period that begins on a Friday and continues Monday through Friday of the following week. On four of those days, you will have exposures to chloropicrin or air. On all 6 days, you will have measurements performed on your nose, eyes, and breathing. These will include taking samples of tears and mucus, photographing your eye, and measuring a vapor from your mouth. The urine test for pregnancy-status will be repeated when more than a week has gone by since your last one.

You will receive $15 per hour for screening and subsequent testing. If you complete all testing, you could earn an amount in the vicinity of $700. There will be no

direct benefits to you of participation in this research. However, the investigators may learn more about how to set standards for health regarding exposure to chloropicrin.

We will advise you of any significant research findings relevant to your continued participation.

You will experience some discomfort just by the nature of the work you will perform. The discomfort you can anticipate is some irritation in the nose, throat, and eyes that could be sharp enough to cause blinking and tearing. Exposure to chloropicrin in amounts greater than anticipated in the studies have resulted in temporary tearing and painful stinging eyes and nausea and vomiting that are completely reversible after the exposure. We expect the discomfort to be short lasting. You will be free to discontinue exposure at any instant. If you in the middle of an exposure and wish to stop, a staff member will guide you out of it. Because you will be participating in an experiment, we must apprise you that there may be some risks that are currently unforeseeable.

Although we will not accept into the study persons with any known tendency to develop an asthmatic reaction (difficulty breathing) from exposure to a vapor, we will be watchful for any such reaction in those who are accepted and will explain to you how to report such an event. If such a reaction should occur to you during a session, we will have a trained person available to manage your symptoms under medical supervision. This person will consult immediately with Dr. Thomas Bruff, one of our physician-investigators. If such a reaction should occur after you have left, page Dr. Bruff directly (619-407-1606).

Participation in research is entirely voluntary. You may refuse to participate or withdraw at any time without jeopardy to the medical care you will receive at this institution. We may terminate your involvement if we find that you are unable to meet our schedule, e.g., if you come late or fail to show up for scheduled sessions.-

If you are injured as a direct result of participation in this research, the University of California will provide any medical care needed to treat those specific injuries. The sponsor, Chloropicrin Manufacturers Task Force, will pay all reasonable medical and hospital costs required for diagnosis and treatment of those injuries specifically caused by the research. However, neither the University nor the sponsor will provide any other form of compensation if you are injured. You may call the Human Research Protections Program at (858) 455-5050 for more information about this, to inquire about your rights as a research subject, or to report research-related problems.

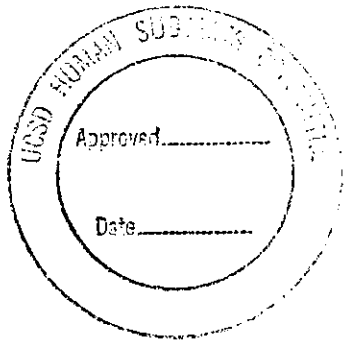
_____ has explained the study and answered your questions. If you have other questions or research-related problems, you may reach Dr. Cain at (858) 622-5830.

No personal identifiers will be released in any public document or report. Federal and state regulators, the UCSD Institutional Review Board, and the study sponsor will have access to the raw data. The raw data may be subject to release by court order.

By signing you indicate that you have read, understand and considered all of the terms in this form, the attached Human Subjects Bill of Rights and information presented to you, and that you have asked and have had specifically answered any questions that you have regarding this research program. You will be given a copy of this consent document and a copy of "The Experimental Subject's Bill of Rights" to keep.

You agree to participate.

_____	_____	_____
Subject's signature	Witness	Date



Invitation to Participate in Three-Week Study at the Chemosensory Perception Laboratory

The Material

We are running a new phase of a study of the vapor chloropicrin. Many of you have participated in earlier phases. This phase requires quite a commitment of time, but we hope that some of you will have that time. First let me say something about chloropicrin. It is an irritating vapor, particularly to the eyes. We are studying exposures like those that occur to agricultural workers in California, particularly workers involved in planting strawberries. Chloropicrin has been used along with the fumigant methyl bromide to prepare fields for planting. Methyl bromide does not signal its presence by sensory cues. Chloropicrin does, so it warns of the presence of methyl bromide. Chloropicrin is also used to warn people of the presence of termite treatment in tented houses.

The Timing

The final phase of our work involves repeated exposures in periods of four days. These always occur on Mondays, Tuesdays, Wednesdays, and Thursdays, in a row. On the previous Friday, we need to take pre-exposure measurements. On following Friday, we need to take post-exposure measurements. This means that a full block of days comprises Friday, Monday, Tuesday, Wednesday, Thursday, and Friday, a total of six. We need to do this three times for each subject. That adds up to 18 days. As you can see, since we begin on Friday and end on Friday, you cannot participate in successive weeks. You need to have at least a week in between.

The study will run into June, which leaves time for people to get in three blocks at a reasonable pace. I would prefer, however, that you choose alternating weeks and have arranged the schedule to favor this.

Within a block of six days, you would need to devote two hours on the Fridays and up to 3.5 hours on the Monday, Tuesday, Wednesday, and Thursdays. On the weekdays of exposure, the actual exposure will last one hour. The rest of the time you would have pre-exposure or post-exposure testing or would just relax. There is a one-hour period after the exposure that involves no testing.

The Pay

The pay will equal $15 per hour, which you will receive at the end of each six-day block. If a block requires 18 hours, as we anticipate, you would make $270. Over the three blocks, you would make $810.

The Rules

If you decide to participate, you should intend to finish. If you do not complete all eighteen days, we will need to discard your results and run someone else in your place. As always, you will be free to leave the study at will, but we hope that this occurs only if you truly cannot continue.

What happens if you find that you are unexpectedly unable to participate on a given day? We would need to reschedule you for a later week because the design of the study requires that your exposures occur on successive days. So, if you got halfway through a

week and some emergency occurred, we would need to start you in another week. You would, however, get paid for the time spent until your emergency and you would be paid again for the rescheduled week. We never use money as a stick, only as a carrot.

Aside from any financial considerations, we need to see this study finish on time because the results will be submitted in July to the State of California for consideration in occupational and environmental rule-making. (As I have mentioned to some of you previously, our [your] data on glutaraldehyde and on mineral dusts have been submitted to health authorities around the world for consideration in rule-making.)

The Schedule

You will see below a calendar of six-day blocks arranged into four sets. Ideally you could fit into one set. If you take a look at Set 1, you will see that Subject 3 (S3), for example, will come in March 19 at 11:00 AM for his/her first session. That session will last two hours. S3 will return Monday (3/22), Tuesday (3/23), Wednesday (3/24), and Thursday (3/25) at 10:00 AM for sessions that will last 3.5 hours each. S3 will return again on Friday (3/26) at 11:00 for a final two-hour session of that block. If things went according to schedule, S3 will have worked 18 hours and will collect $270 on Friday, March 26th. If testing took longer, S3 would make commensurately more.

If S3 can stay within set 1, he/she would return April 2 for a second block of six sessions and then again April 16 for a third set. However, if S3 cannot make those times, he/she can seek other places in the schedule. I cannot guarantee complete flexibility, but I will do everything I can to give everyone what he/she wants.

Those of you at UCSD will recognize that the beginning of the study corresponds to the Friday of finals week, leading into your break. For those staying in San Diego, this would be a good week to start.

Two other notes on the schedule: 1) There is one exception to the Friday-to-Friday rule, namely in the block beginning May 28, where we will skip Monday (Memorial Day), but run Tuesday to Saturday. 2) Initials in a box mean that someone has already taken the slot.

Finally, we have screened most of you for the chloropicrin study and will need only to update the status of your health. If we have not screened you, we will need to do so. If you wish to participate, please let me know by e-mail (wcain@ucsd.edu) and include a phone contact since we may need to speak about your screening. When you contact me by e-mail, please let me know what slots you would prefer.

If you are interested, try to act fast so you can have your preferences. We can negotiate modifications later.

Thanks,

William S. Cain, Professor of Surgery (Otolaryngology), UCSD

Set 1

1st Week	Fri 19-Mar	Mon 22-Mar	Tues 23-Mar	Wed 24-Mar	Thu 25-Mar	Fri 26-Mar
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM	CP	CP	CP	CP	CP	CP
9:30 AM		VL	VL	VL	VL	
10:00 AM	VL	S3	S3	S3	S3	VL
10:30 AM		S4	S4	S4	S4	
11:00 AM	S3					S3
11:30 AM						
12:00 PM	S4					S4
12:30 PM						
1:00 PM	S5					S5
1:30 PM		S5	S5	S5	S5	
2:00 PM	S6	S6	S6	S6	S6	S6
2:30 PM		S7	S7	S7	S7	
3:00 PM	S7	S8	S8	S8	S8	S7
3:30 PM			S8	S8	S8	
4:00 PM	S8					S8
4:30 PM						
5:00 PM						
5:30 PM						

Set 2

1st Week	Fri 26-Mar	Mon 29-Mar	Tues 30-Mar	Wed 31-Mar	Thu 1-Apr	Fri 2-Apr
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM		S9	S9	S9	S9	
9:30 AM	S9	S10	S10	S10	S10	S9
10:00 AM		S11	S11	S11	S11	
10:30 AM	S10	S12	S12	S12	S12	S10
11:00 AM						
11:30 AM	S11					S11
12:00 PM						
12:30 PM	S12					S12
1:00 PM						
1:30 PM	S13	S13	S13	S13	S13	S13
2:00 PM		S14	S14	S14	S14	
2:30 PM	S14	S15	S15	S15	S15	S14
3:00 PM		S16	S16	S16	S16	
3:30 PM	S15					S15
4:00 PM						
4:30 PM	S16					S16
5:00 PM						
5:30 PM						

2nd Week	Fri 2-Apr	Mon 5-Apr	Tues 6-Apr	Wed 7-Apr	Thu 8-Apr	Fri 9-Apr
	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM	S1	CP	CP	CP	CP	S1
9:30 AM		DP	DP	DP	DP	
10:00 AM	DP	S3	S3	S3	S3	DP
10:30 AM		S4	S4	S4	S4	
11:00 AM	S3					S3
11:30 AM						
12:00 PM	S4					S4
12:30 PM						
1:00 PM	CP					CP
1:30 PM		S5	S5	S5	S5	
2:00 PM	ML	ML	ML	ML	ML	ML
2:30 PM		S7	S7	S7	S7	
3:00 PM	S7	S8	S8	S8	S8	S7
3:30 PM			S8	S8	S8	
4:00 PM	S8					S8
4:30 PM						
5:00 PM						
5:30 PM						

2nd Week	Fri 9-Apr	Mon 12-Apr	Tues 13-Apr	Wed 14-Apr	Thu 15-Apr	Fri 16-Apr
	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM		S9	S9	S9	S9	
9:30 AM	S9	S10	S10	S10	S10	S9
10:00 AM		S11	S11	S11	S11	
10:30 AM	S10	S12	S12	S12	S12	S10
11:00 AM						
11:30 AM	S11					S11
12:00 PM						
12:30 PM	S12					S12
1:00 PM						
1:30 PM	S13	S13	S13	S13	S13	S13
2:00 PM		S14	S14	S14	S14	
2:30 PM	S14	S15	S15	S15	S15	S14
3:00 PM		S16	S16	S16	S16	
3:30 PM	S15					S15
4:00 PM						
4:30 PM	S16					S16
5:00 PM						
5:30 PM						

3rd Week	Fri 16-Apr	Mon 19-Apr	Tues 20-Apr	Wed 21-Apr	Thu 22-Apr	Fri 23-Apr
	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM	S1	CP	CP	CP	CP	S1
9:30 AM		DP	DP	DP	DP	
10:00 AM	DP	S3	S3	S3	S3	DP
10:30 AM		S4	S4	S4	S4	
11:00 AM	S3					S3
11:30 AM						
12:00 PM	S4					S4
12:30 PM						
1:00 PM	CP					CP
1:30 PM		S5	S5	S5	S5	
2:00 PM	ML	ML	ML	ML	ML	ML
2:30 PM		S7	S7	S7	S7	
3:00 PM	S7	S8	S8	S8	S8	S7
3:30 PM			S8	S8	S8	
4:00 PM	S8					S8
4:30 PM						
5:00 PM						
5:30 PM						

3rd Week	Fri 23-Apr	Mon 26-Apr	Tues 27-Apr	Wed 28-Apr	Thu 29-Apr	Fri 30-Apr
	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM		VL	VL	VL	VL	
9:30 AM	VL	S10	S10	S10	S10	VL
10:00 AM		S11	S11	S11	S11	
10:30 AM	S10	S12	S12	S12	S12	S10
11:00 AM						
11:30 AM	S11					S11
12:00 PM						
12:30 PM	S12					S12
1:00 PM						
1:30 PM	S13	S13	S13	S13	S13	S13
2:00 PM		S14	S14	S14	S14	
2:30 PM	S14	S15	S15	S15	S15	S14
3:00 PM		S16	S16	S16	S16	
3:30 PM	S15					S15
4:00 PM						
4:30 PM	S16					S16
5:00 PM						
5:30 PM						

Set 3								Set 4						
	Fri	Mon	Tues	Wed	Thu	Fri			Fri	Mon	Tues	Wed	Thu	Fri
1st Week	30-Apr	3-May	4-May	5-May	6-May	7-May	1st Week		7-May	10-May	11-May	12-May	13-May	14-May
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final	Time		Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM	DP	DP	DP	DP	DP	DP	9:00 AM			VL	VL	VL	VL	
9:30 AM	S16	S16	S16	S16	S16		9:30 AM		VL	S26	S26	S26	S26	VL
10:00 AM	S18	S19	S19	S19	S19	S18	10:00 AM			S27	S27	S27	S27	
10:30 AM		S20	S20	S20	S20		10:30 AM		S26	S28	S28	S28	S28	S26
11:00 AM	S19					S19	11:00 AM							
11:30 AM							11:30 AM		S27					S27
12:00 PM	S20					S20	12:00 PM							
12:30 PM							12:30 PM		S28					S28
1:00 PM	S21					S21	1:00 PM							
1:30 PM		S21	S21	S21	S21		1:30 PM		S29	S29	S29	S29	S29	S29
2:00 PM	S22	S22	S22	S22	S22	S22	2:00 PM			S30	S30	S30	S30	
2:30 PM		S23	S23	S23	S23		2:30 PM		S30	S31	S31	S31	S31	S30
3:00 PM	S23	SS	SS	SS	SS	S23	3:00 PM			S32	S32	S32	S32	
3:30 PM							3:30 PM		S31					S31
4:00 PM	SS					SS	4:00 PM							
4:30 PM							4:30 PM		S32					S32
5:00 PM							5:00 PM							
5:30 PM							5:30 PM							

	Fri	Mon	Tues	Wed	Thu	Fri			Fri	Mon	Tues	Wed	Thu	Fri
2nd Week	14-May	17-May	18-May	19-May	20-May	21-May	2nd Week		21-May	24-May	25-May	26-May	27-May	28-May
	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12			Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final	Time		Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM	S17	S17	S17	S17	S17	S17	9:00 AM			S25	S25	S25	S25	
9:30 AM		S18	S18	S18	S18		9:30 AM		S25	S26	S26	S26	S26	S25
10:00 AM	S16	S15	S19	S19	S19	S18	10:00 AM			S27	S27	S27	S27	
10:30 AM		S20	S20	S20	S20		10:30 AM		S26	S28	S28	S28	S28	S26
11:00 AM	S19					S19	11:00 AM							
11:30 AM							11:30 AM		S27					S27
12:00 PM	S20					S20	12:00 PM							
12:30 PM							12:30 PM		S28					S28
1:00 PM	S21					S21	1:00 PM							
1:30 PM		S21	S21	S21	S21		1:30 PM		S29	S29	S29	S29	S29	S29
2:00 PM	ML	ML	ML	ML	ML	ML	2:00 PM			S30	S30	S30	S30	
2:30 PM		S23	S23	S23	S23		2:30 PM		S30	S31	S31	S31	S31	S30
3:00 PM	S23	SS	SS	SS	SS	S23	3:00 PM			S32	S32	S32	S32	
3:30 PM		SS	SS	SS	SS		3:30 PM		S31	S32	S32	S32	S32	S31
4:00 PM	SS					SS	4:00 PM							
4:30 PM							4:30 PM		S32					S32
5:00 PM							5:00 PM							
5:30 PM							5:30 PM							

	Fri	Tues	Wed	Thu	Fri	Sat			Fri	Mon	Tues	Wed	Thu	Fri
3rd Week	28-May	1-Jun	2-Jun	3-Jun	4-Jun	5-Jun	3rd Week		4-Jun	7-Jun	8-Jun	9-Jun	10-Jun	11-Jun
	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18			Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
Time	Initial	Expos.	Expos.	Expos.	Expos.	Final	Time		Initial	Expos.	Expos.	Expos.	Expos.	Final
9:00 AM	S17	S17	S17	S17	S17	S17	9:00 AM			S25	S25	S25	S25	
9:30 AM		S18	S18	S18	S18		9:30 AM		S25	S26	S26	S26	S26	S25
10:00 AM	S18	S19	S19	S19	S19	S18	10:00 AM			S27	S27	S27	S27	
10:30 AM		S20	S20	S20	S20		10:30 AM		S26	S28	S28	S28	S28	S26
11:00 AM	S19					S19	11:00 AM							
11:30 AM							11:30 AM		S27					S27
12:00 PM	S20					S20	12:00 PM							
12:30 PM							12:30 PM		S28					S28
1:00 PM	S21					S21	1:00 PM							
1:30 PM		S21	S21	S21	S21		1:30 PM		S29	S29	S29	S29	S29	S29
2:00 PM	S22	S22	S22	S22	S22	S22	2:00 PM			S30	S30	S30	S30	
2:30 PM		S23	S23	S23	S23		2:30 PM		S30	S31	S31	S31	S31	S30
3:00 PM	S23	SS	SS	SS	SS	S23	3:00 PM			S32	S32	S32	S32	
3:30 PM							3:30 PM		S31					S31
4:00 PM	SS					SS	4:00 PM							
4:30 PM							4:30 PM		S32					S32
5:00 PM							5:00 PM							
5:30 PM							5:30 PM							

Text for **Flyer for Human Sensory Irritation for Chloropicrin**

A. Phases 1, 2, and 3b

Topic: Subjects wanted for participation in research on perception of feel from vapors.

Who: Men and nonpregnant women in normal health, nonsmokers, 18-35years of age, who have had no colds or other infections within the previous month.

What: Subjects will need to give a medical history and go through screening (approximately 1.5 hours, with $30 payment) to establish that their noses, eyes, and airways are healthy. Those who pass screening will be offered the opportunity to participate in a number of sessions in which they will be asked to judge the presence or the intensity of the feel of a chemical vapor. There will be some low-level irritation associated with some exposures. Depending upon the phase of the study, subjects may be asked to some additional testing as occurs in the screening phase. Women will be tested for pregnancy by a urine test.

Where: Chemosensory Perception Laboratory of the UCSD Department of Surgery at the La Jolla Village Professional Center, Suite 1226, 8950 Villa La Jolla Dr.

When: Screening scheduled by mutual convenience.

Payment: $15 per hour. Subjects who pass screening can make $200 to $900 for participation, depending upon the phase.

Contact Kevin at 858-622-5830. Principal Investigator: William S. Cain, Ph.D.

B. Phase 3a

Topic: Subjects with allergic rhinitis, who currently have nasal congestion and runny noses from pollen or other seasonal allergens, wanted for participation in research on perception of feel from vapors.

Who: Men and nonpregnant women in normal health (aside from allergic rhinitis), nonsmokers, 18-35years of age, who have had no colds or other infections within the previous month.

What: Subjects will need to give a medical history and go through screening (approximately 1.5 hours, with $30 payment) to establish that their noses, eyes, and airways are healthy, except for the allergic rhinitis. Those who pass screening will be offered the opportunity to participate in a number of sessions in which they will be asked to judge the feel of a chemical vapor. Some of the testing done in screening will be repeated in connection with the exposures.

Where: Chemosensory Perception Laboratory of the UCSD Department of Surgery at the La Jolla Village Professional Center, Suite 1226, 8950 Villa La Jolla Dr.

When: Screening scheduled by mutual convenience.

Payment: $15 per hour. Subjects who pass screening can make approximately $250.

Contact Kevin at 858-622-5830. Principal Investigator: William S. Cain, Ph.D.

Subjects: $15 per Hour

UCSD Chemosensory Perception Lab

Located behind the Rock Bottom Brewery in La Jolla

We seek to screen healthy subjects for studies on perception and associated effects on the eye, nose, and throat from exposure to vapors. Screening pays $20 and takes about 1.5 hours. Studies pay $15/hour.

A study about to begin needs participants for about 16 hours per week for three non-consecutive weeks. Total pay: $810.

Send e-mail to wcain@ucsd.edu for info about screening and appointments.

Chemosensory Studies
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