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Reregistration Branch 2, Health Effects Division (7509C)
EPA Secondary Reviewer: Judy Facey, Ph.D. **Date**_____
Reregistration Branch 2, Health Effects Division (7509C)

TXR#: 0053064

DATA EVALUATION RECORD

STUDY TYPE: Special Study: Human Eye Irritation and Odor Threshold Non-guideline

PC Code: 081501 **DP BARCODE:** DP312312
Decision No.: 346163

TEST MATERIAL (purity): Chloropicrin (99%)

SYNONYMS: trichloronitromethane, tripicrin,

CITATION: Cain, W.S. (2004) Human Sensory Irritation Testing for Chloropicrin. Chemosensory Perception Laboratory, Department of Surgery (Div. of Otolaryngology), University of California- San Diego. Study Identification: PIC-1 December 14, 2004 MRID 46443801.

SPONSOR: Chloropicrin Manufacturers Task Force

EXECUTIVE SUMMARY: To determine a subjects sensitivity for the detection and characterization of feel to the human eye, nose, and/or throat produced by chloropicrin vapors, as well as the odor threshold, healthy volunteers (18 to 35 years of age, average 23 years) were exposed to a range of vapor concentrations and exposure durations in a controlled laboratory setting (MRID 46443801). The investigation consisted of three phases. Statistical analyses including all subjects for each phase of the study were provided in the study report. U.S. EPA Health Effects Division (HED) analysts provided a logistical regression when appropriate as well as an analysis for only those subjects positively detecting chloropicrin for Phases I and II of the study[1].

Phase I: The objective of Phase I was the identification of chloropicrin by odor (both nostrils, single sniff), eye feel (one eye, 25 seconds), or nasal feel (one nostril, 7 seconds) at 356 ppb, 533 ppb, 800 ppb and 1200 ppb generated from a vapor delivery device. Phase I consisted of 62 subjects (32 male, 30 female) for odor and 63 subjects (32 male, 31 female) for eye feel. The same subjects participated for both odor and eye feel. Confidence of feel was rated 1 to 5, with 1= very low, 3= moderate, and 5= very high confidence. Severity of feel was not rated in Phase I.

An independent HED analysis indicate approximately 13% (8 of 62) of subjects (5/30 female and 3/32 male) failed to detect odor of chloropicrin over the range of concentrations evaluated. Approximately 7 of 63 (11%) subjects failed to detect eye feel (2 male, 5 female) at any concentration. The feel of chloropicrin in the nose was not a reliable endpoint and was

[1]HED analysis of Chloropicrin Human Irritation Study provided by Carol Christensen of the Chemistry Exposure Branch. 2005. See Addendum #1.

therefore dropped prematurely from the phase by the study director. When subjects less sensitive to the detection of odor or eye feel of chloropicrin were removed from the analysis, 70% (38 of 54) and 34% (19 of 56) of subjects positively identified odor and eye feel, respectively, initially at the lowest concentration (356 ppb) and consistently to the highest concentration (1200 ppb). The median for subjects detecting odor is 356 ppb while eye feel is between 356 ppb and 533 ppb.

The median concentration for all subjects at which the odor of chloropicrin was detected 50% of the time was 700 ppb or 590 ppb for males and 810 ppb for females. The calculated median for all subjects for ocular feel was 900 ppb or 790 ppb males and 1010 ppb females.

Summary Table 1. Phase 1 results for odor and eye detection of chloropicrin

Odor Detection				
Exposure Concentrations	**Exposure Duration**	**# of subjects**	**Study Report (Group)Analysis** **Odor Detection and Median**	**HED Analysis (Detecting Subjects only)**
0, 356, 533, 800 or 1200 ppb	"sniff"	62 (32 male, 30 female)	Odor Median = 700 ppb (590 ppb M and 810 F)	70% odor detection at all levels 13% failed to detect (8 of 62) EC10=215 ppb EC50=406 ppb Median= 356 ppb
Eye Detection				
Exposure Concentrations	**Exposure Duration**	**# of subjects**	**Study Report (Group) Analysis** **Eye Detection and Median**	**HED Analysis (Detecting Subjects only)**
0, 356, 533, 800 or 1200 ppb	25 seconds	63 (32 male, 31 female)	Eye Median = 900 ppb (790 ppb M and 1010 ppb F)	34% eye detection at all levels 11% failed to detect EC10= 38 ppb EC50= 242 ppb Median between 356 and 533 ppb
Examination of nasal feel was terminated by the study director in Phase I due to lack of subjects responding to nasal feel. data taken from MRID 46443801				

For Phase I, approximately 10% to 13% of subjects failed to detect either odor or eye feel after momentary exposures to chloropicrin over the range evaluated. The median concentration for all subjects for odor detection is 700 ppb, 590 ppb males and 810 ppb females. The median concentration of all subjects for detection of eye feel is 900 ppb, or 790 ppb males and 1010 ppb females. The median for only those subjects detecting odor is 356 ppb while eye feel is between 356 ppb and 533 ppb.

Phase II Design: The purpose of Phase II was the detection of chloropicrin in the eyes, nose, and/or throat during exposure to 50 ppb, 75 ppb, 100 ppb, or 150 ppb chloropicrin vapor in a walk-in chamber for 20-30 minutes. A total of 62 subjects (32 male, 30 female) participated in Phase II. 12 of 30 female subjects and 14 of 32 male subjects also participated in Phase I of the study. Subjects responded "yes" for a positive feel or "no" for no feel. A level of confidence to each event (eye, nose, throat) was also recorded with 1= not certain, 2= moderately certain, and 3= very certain. Odor was not a parameter in Phase II. The first exposure in a day consisted of a known blank (air). This exposure served to acclimate the subjects to the task in the chamber. The subjects were asked to perform ratings as they would for future blinded exposures.

One female subject left the exposure chamber after 16 minutes at 75 ppb. An explanation for this subject's premature exit from the chamber was not provided. At 150 ppb, this same subject along with another male in the chamber left the chamber after 15 mintues. On a separate day of testing, one female and one male subject also left the exposure chamber after 15 minutes of exposure to 150 ppb. Again, no explanation was given for these subjects's premature departure from the chamber. No subjects left the chamber at 50 ppb or 100 ppb.

As was the case in Phase I, results of Phase II indicate eye feel was more sensitive than either nose or throat feel. ANOVA results provided in the study report indicated concentration and duration was significant ($p<0.0001$) for the eye only. For example, as a group, subjects differentiated 50 ppb chloropicrin in the eyes from the blank after 20 minutes of exposure. Differentiation from blank occurred after 5 minutes at 75 ppb, 3 minutes at 100 ppb, and 2 minutes at 150 ppb. There was no significant interaction with sex for the eyes, nose, or throat.

On an individual basis, binary detection indicators (yes/no) developed by HED analysts were combined by participant across dose levels. Using eye feel as a marker of detection of the chemical, 20 of the 62 participants (32%) could not detect chloropicrin at any concentration. Specifically, 12 of 30 (40%) females and 8 of 32 (25%) males failed to make progress toward eye detection over a 30 minute period of exposure. In addition, 46 (74%) and 48 (77%) subjects among 62 could not detect the chemical via the nose or throat, respectively at any concentration.

Subjects less sensitive to eye feel from chloropicrin exposure were then omitted from the HED analysis. By removing the subjects not detecting chloropicrin over the ranges evaluated, the variability of those subjects positively detecting chloropicrin was analyzed (N=42). Subjects who rated high confidence (5 or 6) for eye feel at least twice during the 20 to 30 minute exposure were included in the analysis. With these criterion, 16 (8 male, 8 female) of 42 (62-20=42) or 38% initially and consistently detected chloropicrin at 50 ppb. Simple tally methods resulted in a median detection level of 75 ppb. Similarly, a total of 5 of 42 subjects (3 male, 2 female or 12%) initially detected at 75 ppb while 11 of 42 subjects (8 male, 3 female or 26%) detected starting at 100 ppb, and 10 of 42 subjects (5 male, 5 female or 23%) detected only at 150 ppb.

Summary Table 2. Tabulation of eye irritation confidence from participating subjects in phase 2

Confidence of Eye Irritation					
Exposure Concentrations	**Exposure Duration**	**# of subjects**	**Exit from Chamber**	**Study Report (Group) Results**	**HED Analysis (Detecting Subjects Only)**
0, 50, 75, 100, 150 ppb	20-30 minutes	62 32 M, 30 F	- 75 ppb: 1 F after 16 minutes - 150 ppb: 4 subjects (2M,2F) after 15 minutes	ANOVA: P<0.0001, level and duration in eye	38% initially & consistently detected at 50 ppb (16 of 42) Ocular Median 75 ppb
data taken from MRID 46443801					

For Phase II, one female subject left at 75 ppb and again at 150 ppb with another male. On a separate testing day, one female and male left the chamber prematurely at 150 ppb. Thirty-eight percent (8 males and 8 females) of subjects, detected chloropicrin initially at 50 ppb and consistently up to 150 ppb.

Phase III Design: The goal of Phase III in this study was the detection of chloropicrin vapor as evidenced by irritation to the eyes, nose, and/or throat after 1 hour (60 minute) exposures repeated over 4 consecutive days. Concentrations tested included blank (air), 100 ppb, and 150 ppb. This phase included a clinical exam of the eyes, nose, and throat, as well as pulmonary function testing with the outcome variable FEV_1 (Forced Expiratory Volume), and FVC (Forced Vital Capacity), rhinomanometry, and nasal cytology. In addition, an assessment was performed based on ocular cytology from samples of cells taken from the conjunctival membrane inside the lower eyelid and from the concentration of exhaled nitric oxide sampled from the lung (eNO) and nose (nNO). Odor was not measured in Phase III.

Subjects participated in 3 cycles (6 days per cycle) of 6 sessions, each beginning on Friday and ending on Friday (no measurements taken on Saturday or Sunday). Subjects participated in exposure chambers for 1 hour each on Monday through Thursday (4 consecutive days). The 3 cycles included exposure to 100 ppb, 150 ppb, and just air (blank). The order in which the subject was exposed to these concentrations was random to prevent confounding order with level. At least one week separated the end of one block and the beginning of another for a subject.

Subjects rated their symptoms in three setting: 1) severity of effect for eye, nose, and throat while in the chamber (0= no symptom to 3= severe); 2) before and after exposure in the chamber and at the beginning and end of each week of exposures; and 3) at the beginning and end of a cycle of exposures, via the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), a series of 28 questions in seven domains, where the subjects used a seven point scale from Not Troubled to Very Troubled. The first two instruments referred to how the subject felt at the time of rating, the RQLQ referred to how the subject felt over the previous week.

When in the exposure chamber, subjects rated symptoms (0 to 3) after 30 seconds, at 1 minute, and every minute until the end of the exposure at 60 minutes. Every 10 minutes study

personnel read and recorded the subject's blood oxygen saturation from a pulse oximeter attached to the finger (data not included in report).

A total of 15 males and 17 females participated in Phase III. Two females in Phase III also participated in Phases I and II. One male in Phase III also participated in Phases I and II and one male in Phase III participated in Phase II only.

Subjects gave higher ratings to symptoms in the eye than to those in the nose and throat. Subjects gave nominally slightly higher ratings in the nose than in the throat, but expressed no symptoms of consequence at either site. There was no indication of intensification of symptoms based on subject scoring for any parameter on the consecutive days of exposure. For the eye, the study report ANOVA indicated Level ($p<0.001$), and interaction of Level by Duration was significant ($p<0.001$). ANOVA also revealed an effect of Level by Day ($p<0.02$).

As a group with all subjects included (even those not feeling), the analysis provided in the study indicate the average rating of eye irritation at 100 ppb reached approximately 0.5 (1=mild) with 30 minutes to reach steady state, which remained until the final minutes and then sometimes regressed. At 150 ppb, the average rating of eye irritation reached 1 (mild, symptom present, but minimal awareness, easily tolerated) with 20 minutes to steady state until fading slightly at the final minutes.

On an individual level, the severity of ocular irritation reported by subjects in Phase III varied from no symptoms to severe at both 100 ppb and 150 ppb. 5 of 17 females (29%) and 7 of 15 males (47%) rated no eye irritation at 100 ppb while 3 of 17 females (18%) and 5 of 15 males (33%) rated no eye irritation at 150 ppb. Nasal and throat irritation was never reported above a "2" and mainly consisted of "0" or "1".

Scores of severe "3" ocular irritation were sporadic during the first 30 minutes of exposure in 2 females and in 4 males at 100 ppb. The second half of the exposure to 100 ppb (31-60 minutes) revealed a more consistent response in ocular severity (3 females, 5 males). Severe was defined as "symptom hard to tolerate and can interfere with activities of daily living or sleeping". At 150 ppb, 4 females and 3 males reported consistent severe eye irritation beginning as early as 8 to 9 minutes of exposure until the end of exposure at 60 minutes.

Moderate "2" eye irritation was also reported sporadically during the first 30 minutes by the same individuals reporting severe eye irritation but with a more consistent response in moderate eye irritation during the second half (31-60 minutes) of exposure. Two additional females and two additional males reported moderate eye irritation during the second half of exposure that did not report eye irritation during the first half of exposure.

Results for the daily measurements (Cochran Q test) provided in the study indicated the number of times a rating post-exposure exceeded a rating pre-exposure for nasal congestion was not significant ($Q=0.75$) while eye irritation (redness) was significant ($Q=28.8$, $p<0.001$). Nasal congestion and ocular erythema (redness) occurred more than the trivial frequency. However, according to the report, the ocular irritation did not translate into more prominent redness.

No biologically significant changes were observed for the lower respiratory variables. For the lower respiratory variables (FVC, FEV_1, eNO), ANOVA analysis from the study report indicated a significant interaction of Level by Order ($p<0.05$) for FVC (Forced Vital Capacity), with only 3%

variation in FVC. FEV_1 (Forced Expiratory Volume), averaged 93.6% before exposure and 93.7% after exposure. The variation spanned 3% with no significance achieved. Exhaled nitric oxide by the lungs (eNO) equaled 37.8 before exposure and 39.2 after exposure with no significance achieved. Sex was not significant for any of interactions of the three lower respiratory variables.

Two upper respiratory alterations, nNO (nasal nitric oxide) and flow, were observed for one-hour exposures that occurred only day by day. For the upper respiratory variables (nNO, inspiratory flow, expiratory flow), nNO was significant for Level by Order with 399 ppb before exposure and 425 ppb after exposure ($p=0.012$). Level by Order by Day was not significant. nNO increased 1% after exposure to blank, 10% after exposure to 100 ppb, and 8% after exposure to 150 ppb. The effect of nNO did not continue from one day to the next.

Inspiratory flow and expiratory flow equaled 450 and 415 mL/sec, respectively, before exposure and 435 and 406 mL/sec, respectively, after exposure. Chloropicrin had a differential effect on flow. Level by Order was nearly significant ($p=0.087$) with Level by Order by Day not significant. Flow decreased 2% after exposure to blank and increased 2% after exposure to 100 ppb chloropicrin, however, flow decreased by 8% after exposure to 150 ppb. Sex was not significant in any of the relevant interactions for the upper respiratory variables. Physiological effects such as changes in nNO and flow rate may indicate signs of nasal congestion and engorgement.

Cell types and cell numbers from the Rhinoprobe samples (nose or eye) were approximately the same at the end of the cycle as at the beginning. For the RQLQ questionnaire results, nasal congestion was the only parameter that reached a level where more than half of the subjects gave a response above zero. 53% of subjects reported a non-zero response to congestion after 4 days of exposure to the blank vs. 41% and 34% after exposures to 150 ppb and 100 ppb, respectively. The average ratings equaled 0.53, 0.34, and 0.41 for the blank, 100 ppb, and 150 ppb, respectively, where a rating of 1 signified “hardly troubled at all”. Watery eyes, sore eyes, and swollen eyes were scored higher by subjects after exposure to either 100 or 150 ppb chloropicrin than to the blank. The Q test revealed significance for the sore eyes only ($p<0.05$). The highest rating given after exposure to swollen eyes was 0.47.

Summary Table 3. Average group responses (all subjects) to chloropicrin in Phase 3 for chamber exposures at 1 hour periods over 4 consecutive days

Exposure	Definition	Ocular Avg Rating	Time to Recognition	Nasal Avg Rating	Time to Recognition	Throat Avg Rating	Time to Recognition
blank	No symptom	0.1	NA	0.1	5 minutes	0.1	5 minutes
100 ppb	Between no symptom and mild symptom	0.5	30 minutes	0.1	5 minutes	0.1	5 minutes
150 ppb	Mild; Symptom present, but minimal awareness, easily tolerated	1	20 minutes	0.2	5 minutes	0.1	5 minutes
Data taken from final study report pg 67 and Figure 20 (pg 69). NA = not available.							

The LOAEL is 100 ppb, the lowest concentration tested, based on eye irritation, increased nasal nitric oxide (nNO), and differential effect on inspiratory and expiratory flow. A NOAEL was not established in Phase III.

Overall Summary of the Chloropicrin Human Study:

In summary, the concentrations and durations explored in each of the three Phases of this study failed to identify a level at which none of the subjects responded to either irritation or odor of chloropicrin. Likewise, the concentrations and durations were not sufficient to produce a response in all of the subjects during any phase of the study. Therefore, this study does not provide a concentration or duration where none of the healthy individuals were responsive or all healthy individuals detected chloropicrin. This study provides information as to the variability of the responses of healthy individuals to a 'sniff' scenario versus the variability of responses over time and concentration and with repeated exposure to chloropicrin.

At short "sniff" or momentary exposures, a majority of the individuals identifying the odor of chloropicrin did so initially at the lowest concentration (356 ppb) and consistently to the highest concentration (1200 ppb). The calculated median of those subjects detecting odor was 356 ppb. The median concentration for ocular irritation for momentary exposures was between 356 ppb and 533 ppb. However, ocular irritation was detected by some individuals at much lower concentrations but only during longer durations, namely 20 to 30 minutes. Results of this study indicate eye irritation may occur before odor detection for some individuals. However, the variability of the subject's responses to chloropicrin was large. Approximately 30% of subjects did not report eye feel from chloropicrin at the highest dose tested (150 ppb) for 30 to 60 minutes (Phase III). Individuals not detecting chloropicrin at the lower concentrations would not have an early indication of exposure to chloropicrin and unknowingly may remain in an exposure scenario. The individuals more sensitive to the irritating effects of chloropicrin in the eye might be more prone to leaving the area of the chloropicrin exposure.

Summary Table 4. Summary of responses from participating healthy subjects to chloropicrin for odor detection and eye, nose, and throat irritation

Exposure Concentration	Concentrations	Exposure Duration	NOAEL/Threshold	LOAEL/Threshold	Effect
Phase I	0, 356, 533, 800 or 1200 ppb	"sniff"	Study Report Analysis: median odor threshold: 700 ppb M=590 ppb and F=810 ppb median eye irritation: 900 ppb; M=790 ppb and F=1010 ppb No localization of the throat; >1200ppb Independent HED Analysis: 50^{th} percentile for Odor: 356 ppb 50^{th} percentile for Eye: >356 but < 533ppb		
Phase II	0, 50, 75, 100, or 150 ppb	20-30 minutes	One subject left at 75 ppb; 4 subjects left at 150 ppb	38% initially and consistently detected chloropicrin at 50 ppb (16 of 42)	ocular and nasal detection of sensitive individuals
Phase III	100 or 150 ppb	60 minutes/day for 4 consecutive days	Not Established <100 ppb	100 ppb	ocular irritation, increased nasal nitric oxide, differential inspiratory and expiratory flow, 30 minutes to steady state
data taken from MRID 46443801					

This multi-phase sensory irritation study is classified as Acceptable/Non-guideline.

COMPLIANCE: Signed and dated GLP, Internal and External Quality Assurance, and No Data Confidentiality statements were provided.

It should also be noted that a benchmark concentration (BMC) modeling and categorical regression analysis by TERA was submitted to the Agency (MRID 46614801) on behalf of the Chloropicrin Manufacturers Task Force. This analysis relied on the chloropicrin human data (ocular irritation) from phase 3 of the study to establish a BMCL for acute exposure to chloropicrin. Please refer to the review document (TXR. 005422, DP305307) for more details.

I. Materials:

Test Material:	Chloropicrin (99%)
CAS No.:	76-06-2
Manufactured	(4/28/2000) by Trinity Manufacturing (Hamlet, NC) with expiration date of August 2006
Lot No.:	0-120A (pg 355 of report)
Stability:	stable under normal temperatures and pressure
Molecular Weight:	164.39
Specific gravity:	1.65 at 20EC
Purity:	Text indicates 100%, MSDS indicates >99.0%, No formal purity report given Two separate shipments were received of chloropicrin (1/17/2002 and 5/20/2004)
Description:	Colorless liquid with an intensely irritating odor

Diluent Gases: n-Heptane (99%) and Nitrogen

Nitrogen was used as a diluent gas and was not analytically characterized nor were records maintained according to GLP requirements. The n-heptane was used as a diluent in Phase 2 and was used on the basis of a certificate of analysis from the supplier and not further characterized under GLP conditions. Records of standard preparation were also not maintained, but chromatograms exist that show standards were run. The final report indicates that all other analytical work met requirements and the absence of some documentation is not expected to have any impact on the quality, integrity, or results of the study.

II. Study Rationale for Concentration and Duration:

The study report cited Krieger (1996) as a review of the risks to workers from exposure to chloropicrin in agricultural applications. It appears that this reference was relied upon for basing concentration and duration for the human sensory study. From this reference, a time-weighted average of 0.1 ppm (100 ppb) was indicated to evoke no response in humans. The report then indicated concentrations of 0.15 to 0.3 ppm would evoke concentration-dependent sensory detection via chemesthesis, as well as reflex tearing and cough. Concentrations above 0.3 ppm would evoke an increasing degree of irritation. Odor was noted as occurring at about 0.9 ppm.

The study report highlighted the duration was focused on very brief exposures (phase 1) and in more extended exposures (phase 2). The extended phases were focused on concentrations of likely occupational relevance, both below and just above 100 ppb, the ACGIH (American Conference of Governmental Industrial Hygenists) threshold Limit Value (TLV) and OSHA Permissible Exposure Limit (PEL).

III. Subjects: Healthy Human Volunteers:

Total of Individuals:	127 total (majority of subjects recruited from college campuses)
Age:	18 to 35 years (average age 23)
Study Dates:	Study dates not provided in report

Each subject was given a Subject Identification Number (SID) based on date of birth, sequential participation, etc.

IV. Subject's Bill of Rights and Informed Consent:

Subjects received and signed an Experimental Subject's Bill of Rights and Informed Consent Form.

V. Screening of Subjects:

Subjects first answered a questionnaire regarding their health, with focus on airways and eyes (questionnaire provided in Appendix J of report). Signed consent forms, protocols, and Human review Board approval were provided. Only subjects who reported, inter alia, no smoking within a year, no use of recreational drugs within a year, no recent illness, and no history of chronic illness qualified to go on to screening in the laboratory. The only question that subjects could answer "yes" and still remain in consideration for inclusion concerned allergies. Some dormant upper respiratory allergies did not disqualify the subject per se from further consideration. If the person agreed to participate, he/she signed the informed consent form and took a brief odor identification test to assess normality of smell. The standardized test consisted of the odor identification portion of the Connecticut Chemosensory Clinical Research Center (CCCRC) test (Cain, 1989[2]).

A screening packet with forms used to assess the fitness of each participant in the study was generated (packet provided in Appendix J). Packets contained subject contact information, symptom evaluation forms, a pregnancy test for females, and other forms for documentation of screening parameters.

An examination of the nose, throat, and eyes was performed for each participant with any irritation, abnormalities, or abnormal redness scored on 0-3, with a score greater than 1 grounds for exclusion of the study. The examination also included measurement of nasal resistance (Rhino; MultiSpiro or HR-Rhinomanometry) and pulmonary function (MultiSPIRO SX-SILVER). Cells were also taken from the surface of the inferior turbinate of one nasal cavity via a Rhinoprobe scraping.

Criteria for passing screening included the following (pg 42 of final report):

1. On olfactory functioning: identification of 5 or more of test odors of the Connecticut Chemosensory Clinical Research Center Test for odor identification.

2. On clinical exam: Absence of pathology and, in particular, of signs greater than mild.

3. On cytological grounds: Absence of ciliocytophthoria (clumping of chromatin) in epithelial cells as evidence for viral infection, absence of large numbers of neutrophils with intracellular bacteria as evidence of bacterial infection, and absence of large numbers of eosinophils or basophilic cells as evidence of inflammation, all defined by standard criteria.

4. For nasal airway resistance: Absence of clinically abnormal resistance, defined as >5 cm H_20/L/sec for the nostrils combined, a clinical criterion.

[2]Cain WS 1989. Testing olfaction in a clinical setting. Ear, Nose, and Throat Journal, 68, 316-328.

5. For pulmonary function testing: pulmonary function at or above 83% of predicted forced expiratory volume at 1 sec (FEV_1) or forced vital capacity (FVC) for testing by ATS criteria.

VI. Test Apparatus:

A vapor delivery devise (VDD) was used for Phase I of the study. Concentrations for subject exposure were generated by metering either liquid chloropicrin or a chloropicrin-heptane solution into a heated chamber, where it was vaporized and swept by a stream of nitrogen into a Vapor Delivery Devise (VDD, Phase 1), or an environmental chamber (Phases 2 and 3). Figures 1 and 2 (see below) of the final report (pgs 28 and 30) depict a schematic of the VDD and the environment chamber, respectively. Table 4 (pg 27) of the final report listed confirmation data of the stability of detector via liquid injections while Table 5 (pg 31) indicated nominal concentrations for the vapor generation of the three phases of the study.

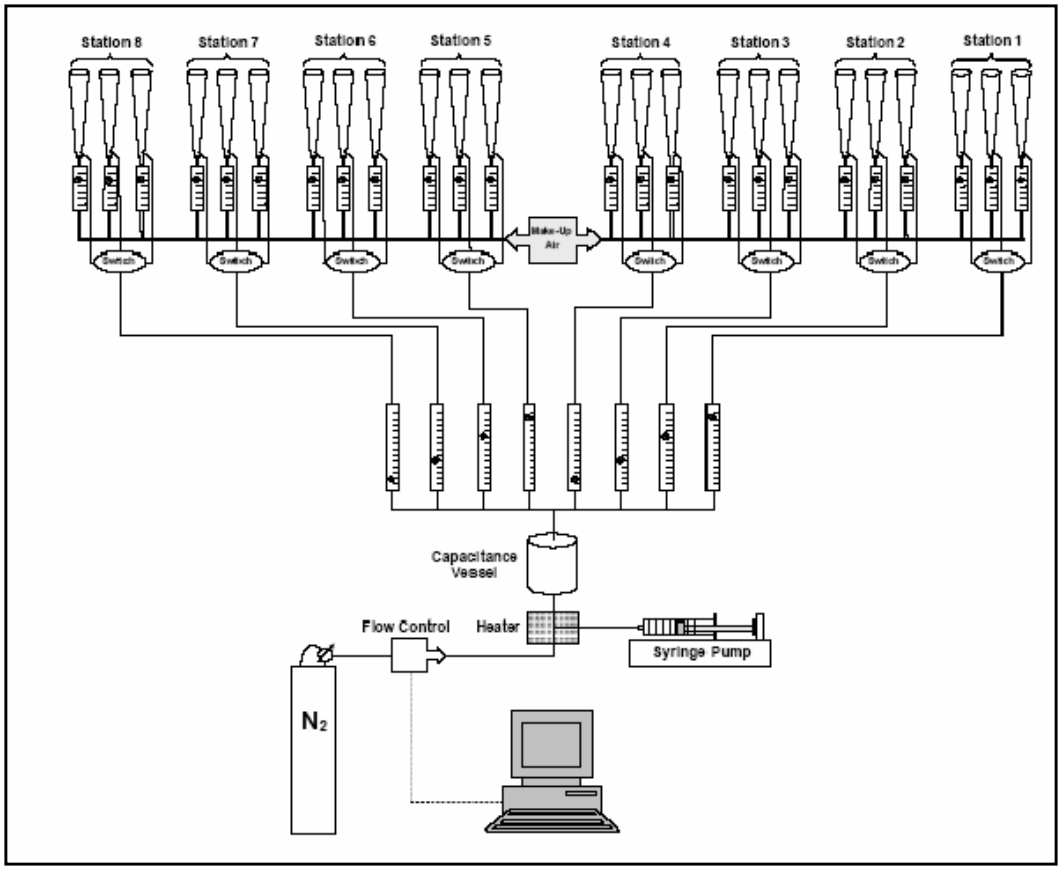

Figure 1. A schematic of the vapor delivery device (VDD). Taken from page 28 of mrid 46443801.

Measurements were also taken from the cones of the VDD (Figure 5 of final report, pg 34, and in Appendix D and E) and indicated the vapor-phase concentration agreed on average with nominal concentration (296 to 1000 ppb) calculated from flows, within 5% (average CV=6.4%). Another set of measurements taken over the nominal range 356 to 1200 ppb found the actual vapor-phase concentration to agree with nominal within 1% (average CV=9.5%).

The height of the cones was adjusted before testing to accommodate all of the subjects in a group. During sniffs in Phase I, the nose of the subject just broke the plane of the face of the cone. For ocular exposure, the subject touched his/her cheekbone to the lip of the cone. This brought the eye essentially to the lip of the cone. Only one eye was exposed at a time. From one station to the next, the subject switched the exposed eye. In addition, each time a subject started a new run, he/she switched which eye was exposed first, in comparison with the eye exposed first from the start of the previous run. The design of the cones insured that the air would wash over the area placed at the face of the cone.

Details of the two environmental chambers were detailed in Appendix F of the final report. The set up that generated vapor in Phase I with the cones also served in Phase 2 and 3 with the environmental chambers. The details of Phase 2 concentrations were presented in Appendix G and Phase 3 in Appendix H.

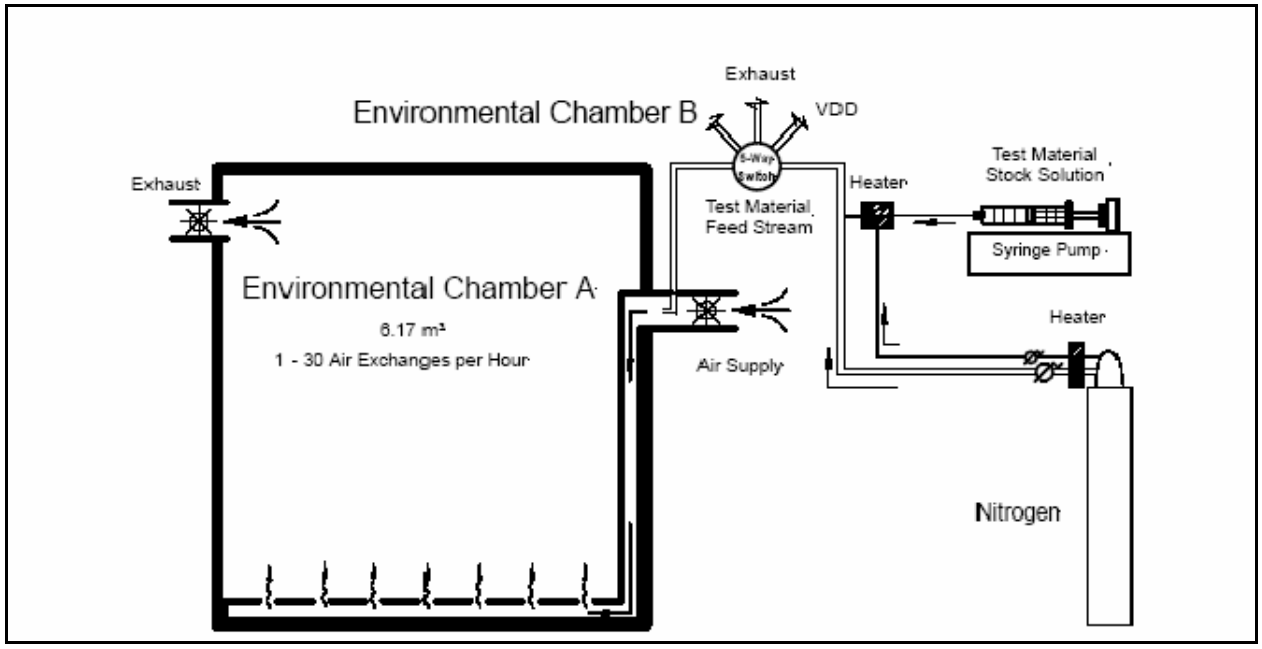

Figure 2. A schematic of Chamber A, with the system to feed test substance and air into the chamber. The feed stream could flow to Chamber B by a change in the position of a valve. Taken from page 30 of mrid 46443801.

For all phases of this study, no positive controls were used. Air was used as a blank.

VII. Test Phases and Results:

Phase I Design (Table 1): The first Phase addressed the issue of detection to brief (momentary) exposures and consisted of three parameters: odor detection, ocular feel, and nasal pungency or feel, which were performed separately. Ideally, a subject remained for a full day of testing, which consisted of up to 30 rounds (e.g., 4 concentrations x 30 rounds = 120 judgments). Testing of odor or feel (eye or nasal) required the subject to decide which of the three cones had the strongest odor or feel among two blanks (air) and one active cone at each station (4 stations total). After the subject sampled each cone, the subject wrote the choice in the appropriate cell of a data sheet carried on a clipboard. The scale of confidence ranged from 1= very low to 5 = very high, with a midpoint of 3 = moderate. The subject completed these judgments before the next trial began. Four trials in the ascending order of concentration completed a round of testing for a subject.

Table 1. Listing of test methods and parameters for Phase 1 of human sensory irritation testing for chloropicrin

Parameters:	Male N	Female N	exposure duration	exposure concentration (ppb)	# of cones	# of concen-trations	# of rounds	Notes
Phase 1	Sensitivity to brief exposures							
odor detection Which cone did you smell odor? 1, 2, or 3? How confident? 1 to 5	32	30	"sniff" (1-2 seconds likely) possible 5 seconds	356, 533, 800, or 1200	3 cones, w/ 1 active only	4	30	1 to 5 scale of confidence (1 very low, 5 very high); no positive or negative controls, random position of active cones Cones adjusted to the nose and eyes of each subject.
ocular detection -nose clips, pick cone 1, 2, or 3? How confident?	32	31	25 seconds	356, 533, 800, or 1200	3 cones, w/ 1 active only	4	30	nose clips to prevent nasal stimulation, no goggles just cones, 1 to 5 scale of confidence (1 very low, 5 very high); no positive or negative controls, random position of active cones Cones adjusted to the nose and eyes of each subject.
nasal detection (left or right nasal)	14	6	"sniff" (1-2 seconds likely) 7 seconds	356, 533, 800, or 1200	1= left 2 = right	4	20	Both nostrils to yokes, but only one nostril exposed to chloropicrin.

Data taken from Appendix K pg 228 of mrid 46443801

Parameter A: The first parameter of Phase 1 examined the level at which the odor of chloropicrin vapor was discernible from a blank (air). The subjects were informed to place their nose to the face of the cone and "sniff". Subjects had a 5 second window of time in which to sniff. The duration of a sniff was up to the subjects, but likely did not exceed 1-2 seconds. Chloropicrin vapor was tested on 62 (32 Males/30 Females) subjects for a possible duration of 5 seconds each to 4 concentrations, starting at the lowest concentration (356 ppb, 533 ppb, 800 ppb, or 1200 ppb).

Study Report Analysis:

On a group level, odor was detected 25% to 60% of the time. A two-way analysis of variance of detection performed by the registrant indicated no statistical significance with concentration and

sex. As a group, the median concentration for the odor of chloropicrin (at a point of 50% detection) was 700 ppb or 590 ppb for males and 810 ppb for females.

Independent HED Analysis:
To determine the distribution of subjects able to detect chloropicrin, HED analysts utilized a method similar to that used in the stats test (e.g., the SAT) to remove the affect of chance or guessing in the enumeration of total number of positive detects (See Addendum 1). A total of 8 (13%) subjects failed to detect odor over the range of concentrations assessed in the first phase of the study. Specifically, 5 of 30 (17%) females and 3 of 32 (9%) males failed to detect the odor of chloropicrin.

In contrast, of those subjects identifying chloropicrin at some level (N=54), 38 of 54 (70%) subjects initially and consistently identified odor beginning at the lowest concentration of 356 ppb to the highest concentration of 1200 ppb. An additional 7 of 54 (13%) identified odor beginning at 533 ppb, 6 of 54 (11%) starting at 800 ppb, and 3 of 54 (6%) at 1200 ppb.

Using a simple tally method, the 50^{th} percentile of the distribution of 'true detectors' is the 27^{th} observation of the 54 persons detecting the chemical, or 356 ppb. Using logistic regression to model the probability of positive detection for odor, the 10^{th} percentile of detection is 215.84 ppb and median is 405.8 ppb. Because the median derived from the logistic operation is based upon the calculated slope of the line, it is slightly different from the median generated from the simple tally method.

Parameter B: The second parameter of Phase 1, ocular detection, was tested on 63 individuals (32 Males/31 Females) for a duration of 25 seconds each to 4 concentrations, starting at the lowest concentration (356 ppb, 533 ppb, 800 ppb, or 1200 ppb). Nose clips were worn in order to prevent nasal stimulation. Only one eye was placed to the face of a cone with the exposed eye being switched from one station to the next. There were an equal number of cones and stations for each eye. As in parameter A, subjects scored their confidence of detection before the next trial began.

Study Report Analysis:
There were no statistical differences between the responses in males compared to females. The median at which subjects detected chloropicrin in the eye 50% of the time, was as a group 900 ppb (males and females), or 790 ppb males and 1010 ppb females.

Independent HED Analysis:
HED analysts determined that 7 subjects failed to detect chloropicrin in the eye over the range of concentrations evaluated in the current study. Specifically, 5 (16%) females and 2 (6%) males failed to detect chloropicrin at any concentration exposure in the eye.

Of the subjects positively detecting ocular feel, 19 of 56 (34%) identified eye feel initially and consistently beginning at the lowest concentration of 356 ppb up to the highest concentration of 1200 ppb. Likewise, 19 of 56 (34%) detected ocular feel beginning at 533 ppb while another 13 of 56 (23%) detected at 800 ppb and 5 of 56 (9%) at 1200 ppb.

Utilizing those subjects positively identifying feel in the eyes, HED analysts determined the median for ocular feel is between 356 ppb and 533 ppb. Using logistic regression, the 10^{th}

percentile is 37.56 ppb and the median is 242.5 ppb. Because the median derived from the logistic operation is based upon the calculated slope of the line, it is slightly different from the median generated from the simple tally method.

Parameter C: The third parameter of Phase 1, nasal detection, was tested on 20 individuals (14 Males/6 Females) for a possible duration of 7 seconds (sniff) each to 4 concentrations, starting at the lowest concentration (356 ppb, 533 ppb, 800 ppb, or 1200 ppb). The subjects were asked to put nostrils to right and left yokes that came from two cones, left and right. Chloropicrin was administered only from one cone and therefore one nostril was exposed, while air came from the other cone or yoke. The subjects were asked to "sniff" and then record nasal feel either in the left or right nostril and scored their confidence before the next trial began. Subjects were cautioned to "sniff" just once.

Only 10% of the subjects detected the feel of chloropicrin in the nose at levels up to 1200 ppb and therefore was not tested further. The table below outlines the details for Phase 1 of the study.

Table 2. Phase 1 results for odor and eye detection of chloropicrin

Odor Detection				
Exposure Concentrations	**Exposure Duration**	**# of subjects**	**Study Report Analysis Odor Detection and Median**	**HED Analysis**
0, 356, 533, 800 or 1200 ppb	"sniff"	62 (32 male, 30 female)	Odor Median = 700 ppb (590 ppb M and 810 F)	70% odor detection at all levels 13% failed to detect (8 of 62 or 3 M and 5 F) EC10=215 ppb EC50=406 ppb Median= 356 ppb
Eye Detection				
Exposure Concentrations	**Exposure Duration**	**# of subjects**	**Study Reported Eye Detection and Median**	**HED Analysis**
0, 356, 533, 800 or 1200 ppb	"sniff"	63 (32 male, 31 female)	Eye Median = 900 ppb (790 ppb M and 1010 ppb F)	34% eye detection at all levels 11% failed to detect (7 of 62 or 2 M and 5 F) EC10= 38 ppb EC50= 242 ppb Median 356 & 533 ppb
Examination of nasal feel was terminated in Phase I due to lack of subjects responding to nasal feel. data taken from MRID 46443801				

For Phase I, approximately 10% to 13% of subjects failed to detect either odor or eye feel after momentary exposures to chloropicrin over the range evaluated. The median concentration for all subjects for odor detection is 700 ppb, 590 ppb males and 810 ppb females. The median concentration of all subjects for detection of eye feel is 900 ppb, or 790 ppb males and 1010 ppb females.

PHASE II Design (Table 3):
The second Phase consisted of a 20-30 minute exposure to chloropicrin vapor in a walk-in chamber at concentrations of 0, 50, 75, 100, and 150 ppb. The number of subjects per day of testing ranged from one to four. On a day with more than one subject, they went through testing simultaneously. This phase examined the subject's response to the feel of chloropicrin in the eyes, nose, and throat over time.

Before the subjects entered the chamber, the investigator explained that the task inside the chamber would entail judgments of confidence that certain sensory events were occurring at closely-spaced periods of time beginning at 30 seconds, then at 1 minute, 2 minute, 3 minute, and so on until the end of exposure. The response was then "yes" for a positive feel or "no" for no feel. The subject needed to attach a level of confidence to each event (nose, eye, and throat). The score rating was 1 = not certain, 2 = moderately certain, 3 = very certain. For example, if a subject detected nothing at all in the nose, the rating was "no" and "3" for no detection and very certain. Additionally, it should be noted that odor was not a parameter evaluated in this phase of the study.

The first exposure in a day consisted of a known blank (air), in order to serve as an acclimation to the task in the chamber (practice run). The subjects made ratings as they would for the subsequent blinded exposures.

Study Analysis for Phase 2: The confidence scores of 1 to 3 were entered as -1 to -3 for "no" judgments and as +1 to +3 for "yes" judgments. This transformed into a scale of 1 to 6, with 1 to 3 describing the -1 to -3 scores, and 4 to 6 describing the +1 to +3 scores, for a total of 6 points. The midpoint of 3.5 indicated the demarcation between "no" and "yes". The scale of 1 to 6 lent itself to ANOVA on the assumption of interval scale measurement.

Table 3. Listing of test periods and concentrations for Phase 2 of human sensory irritation testing for chloropicrin

Parameters:	N (M)	N (F)	Exposure Duration
Sensitivity to 20 or 30 min exposures with blanks in between chloropicrin exposure; "did you feel in the nose, eyes, or throat?"			
blank blinded	32	30	30 min
50 ppb chloropicrin	32	30	30 min
lunch break			45 min
75 ppb chloropicrin	32	30	20 min
blank blinded	32	29	30 min
100 ppb chloropicrin	32	30	20 min
blank blinded	32	30	30 min
150 ppb chloropicrin	32	30	20 min
Number of participating subjects and exposure duration taken from Appendix M of the original report (pgs 261 to 282)			

Phase II Participating Subjects:

12 of the 30 female subjects in Phase II were also in Phase I. The additional 18 female subjects were new to the study in Phase II. Likewise, 14 of 32 male subjects continued from Phase I to Phase II. The additional 18 male subjects were new to the study in Phase II. All subjects participated in each of concentrations scheduled in a day as listed in Table 2.

Phase II Study Report Results:

ANOVA results provided in the study report indicated level and duration of exposure had stronger effects on the eye than on the nose and throat, though significance was achieved for effects on all three parameters ($p<0.0001$). Significance ($p<0.0001$) for the interaction of level by duration was achieved for the eye only. There was no significant interaction with sex for the eyes, nose, or throat.

The feel of chloropicrin in the eye was more sensitive than in the nose or throat, based on confidence scores. The subjects were not asked to rate the severity of the irritation. There was also no data provided for a subjects notation of the detection of odor during the exposure period.

At 50 ppb, none of the subjects (male or female) left the exposure chamber during the 30 minute exposure period. The report analysis indicated the subjects as a group responded with similar ratings for detection in the eye as to the blank for the first 20 minutes, then some differentiation between the blank and chloropicrin. However, it should be noted that on an individual level, 16 subjects initially and consistently detected ocular feel at 50 ppb beginning as early as 3 minutes for some individuals.

At 75 ppb, one female subject left the exposure chamber after 16 minutes. At 75 ppb the group ratings for ocular feel diverged from feel from the blank after 5 minutes.

At 100 ppb, the group responses for ocular feel diverged from blank at about 3 minutes. None of the subjects left the chamber during any time point of the exposure period (20 minutes). In general, the subjects that reported feel in the eye at 50 ppb and 75 ppb also responded to the feel of chloropicrin in the eye at 100 ppb.

At 150 ppb, the group responses for ocular feel diverged from blank responses after 2 minutes. The feel of chloropicrin in the eye was again the most sensitive indicator of exposure. The female that left at 75 ppb also left along with a male subject at 150 ppb. In a separate day of testing, one female and one male left the chamber after 15 minutes. An explanation for the departure from the chamber was not provided in the study report. Dates of testing in the appendices indicate these were the only subjects in the chamber for those days of testing.

Phase II HED Independent Analysis:

On an individual basis, binary detection indicators (yes/no) were combined by participant across dose levels. Using ocular feel as a marker of detection of the chemical, 20 of the 62 participants (32%) could not detect the chemical. Specifically, 12 of 30 (40%) females and 8 of 32 (25%) males failed to make progress toward eye detection over a 30 minute period of exposure. In addition, 46 (74%) and 48 (77%) subjects among 62 could not detect chloropicrin via the nose

or throat, respectively. Subjects not detecting eye feel from chloropicrin exposure were then omitted from the analysis.

For subjects who rated high confidence (5 or 6) for eye feel at least twice during the 20 to 30 minute exposure, 16 of 42 (38%), 8 males and 8 females, initially and consistently detected chloropicrin at 50 ppb to 150 ppb. High confidence scores were reported as early as 3 minutes of exposure. A total of 5 subjects (12%), 3 males and 2 females, initially detected at 75 ppb while 11 subjects (26%), 8 males and 3 females, at 100 ppb and 10 subjects (23%), 5 males and 5 females, at 150 ppb.

Table 4. Tabulation of eye irritation confidence of subjects in phase 2

Confidence of Eye Irritation					
Exposure Concentrations	**Exposure Duration**	**# of subjects**	**Exit from Chamber**	**Study (Group) Report Results**	**HED Analysis (Detecting Subjects Only)**
0, 50, 75, 100, 150 ppb	20-30 minutes	62 32 M, 30 F	- 75 ppb: 1 F after 16 minutes - 150 ppb: 4 subjects (2M,2F) after 15 minutes	ANOVA: P<0.0001, level and duration in eye	38% (16 of 42) initially & consistently detected at 50 ppb Ocular Median 75 ppb
data taken from MRID 46443801					

For Phase II, one female subject left the chamber at 75 ppb and later left the chamber with a male during the 150 ppb period. One female and one male left the chamber in a separate test day at 150 ppb. Thirty-eight percent (8 males and 8 females) of subjects, initially and consistently detected chloropicrin beginning at 50 ppb and up to 150 ppb. These ratings of confidence to the positive detection of eye feel from chloropicrin may serve as a marker for protecting against more serious respiratory outcomes.

PHASE III Design (TABLE 5):
The goal of Phase 3 was the detection of chloropicrin vapor as evidenced by irritation to the eyes, nose, and/or throat after 1 hour (60 minute) exposures repeated over 4 consecutive days. Concentrations tested included blank (air), 100 ppb, and 150 ppb. These concentrations, however, occurred in random order. This phase included a clinical exam of the eyes, nose, and throat, as well as pulmonary function testing with the outcome variable FEV_1 (Forced Expiratory Volume) and FVC (Forced Vital Capacity), rhinomanometry, and nasal cytology. In addition, an assessment was performed based on ocular cytology from cell samples taken from the conjunctival membrane inside the lower eyelid and from the concentration of exhaled nitric oxide sampled from the lung (eNO) and nose (nNO). Odor detection was not measured in Phase III.

Subjects were asked to rate their symptoms in three settings:

1) On a scale of 0 (no symptom) to 3 (severe) for eye, nose, and/or throat irritation while in the chamber;
2) Before and after exposure in the chamber and at the beginning and end of each week of exposures; and
3) At the beginning and end of a cycle of exposures, via the Rhinconjunctivitis Quality of Life Questionnaire (RQLQ), a series of 28 questions in seven domains, where the subjects used a seven point scale from *Not Troubled to Very Troubled* (Juniper and Guyatt, 1991[3]; Juniper, Guyatt, Griffith, and Ferrier, 1996[4]).

The first two instruments referred to how the subject felt at the time of rating, the RQLQ referred to how the subject felt over the previous week.

Subjects served in 3 cycles (6 days per cycle) of 6 sessions, each beginning on Friday and ending on Friday (no measurement on Sat or Sun). Exposures to a given concentration lasted one hour on the four days Monday through Thursday (1 hr exposures for 4 days). The 3 cycles included exposure to 100 ppb, 150 ppb, and just air or blank. The order in which the subject was exposed to these concentrations was random to prevent confounding order with level. At least one week separated the end of one block and the beginning of another for a subject.

When in the chamber, the subject rated symptoms after 30 seconds and then every 1 minute until the end of the exposure at 60 minutes. During this time, the subject remained under scrutiny for level of alertness and general condition.

Irritation severity scoring:
0: 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

[3]Juniper, EF and Guyatt, GH 1991. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clinical and Experimental Allerygy, 21. 77-83.
[4]Juniper, EF, Guyatt GH, Griffith LE, and Ferrie PJ 1996. Interpretation of rhinoconjunctivitis quality of life questionnaire data. Journal of Allergy and Clinical Immunology, 98. 843-845.

The table below summarizes the measurements taken over the six days of a cycle.

Table 5. Listing of test measurements for Phase 3 of human sensory irritation testing for chloropicrin

Measurements:	N (M)	N (F)	Cytology	RQLQ	O2 Saturation	NO	NAR-Spirometry	Symptoms	Clinical Exam
Phase 3	Sensitivity to 60 minute (1 hr.) exposures over 4 days								
Fri: No exposure	15	17	♦	♦				♦	♦
Mon: Pre-exposure	15	17				♦	♦	♦	♦
During Exposure	15	17			♦			♦	
Post-Exposure	15	17			♦	♦	♦	♦	♦
Tues: Pre-Exposure	15	17			♦	♦	♦	♦	♦
During Exposure	15	17			♦			♦	
Post-Exposure	15	17			♦	♦	♦	♦	♦
Wed: Pre-Exposure	15	17				♦	♦	♦	♦
During Exposure	15	17			♦			♦	
Post-Exposure	15	17				♦	♦	♦	♦
Thurs: Pre-Exposure	15	17				♦	♦	♦	♦
During Exposure	15	17			♦			♦	
Post-Exposure	15	17				♦	♦	♦	♦
Fri: No Exposure	15	17				♦	♦	♦	♦

Information about details of the timing of exposure measurements were taken from Figure 11 of the original study (pg 52).

Phase III Participating Subjects:

Of the 17 female subjects in Phase III, only 2 female subjects in Phase III also participated in Phases I and II. Therefore, 15 of 17 female subjects in Phase III were new to the study. One female subject responded with confidence to eye feel at 150 ppb chloropicrin between 12-20 minutes in Phase II and detected odor in Phase I at 533 ppb and above. Another female subject detected eye feel at 50 ppb (sporadically beginning at 10 minutes), 100 ppb (16-20 minutes) and at 150 ppb (4-17 minutes) in Phase II and detected odor in Phase I at 800 and 1200 ppb.

Of the 15 male subjects in Phase III, one male participated in both Phase I and II and one male participated in Phase II only. Therefore, 13 of 15 male subjects were new to the study. One male subject did not detect odor in Phase I but did detect eye feel sporadically at both 50 ppb and 75 ppb, with consistent scores for ocular feel at 100 ppb (15 minutes) and 150 ppb (3

minutes) in Phase II. Another male subject reported eye feel only during the last 3 minutes (18-20 minutes) of exposure to 100 ppb and 150 ppb chloropicrin in Phase II.

The subjects that prematurely left the chamber in Phase II and likely more sensitive to the detection of ocular feel to chloropicrin did not participate in Phase III of the study. Similarly, participants that did not detect irritation from chloropicrin up to 150 ppb in Phase II also did not participate in Phase III of the study.

Phase III Results:

A. Chamber:

Study Report Analysis:

As a group the subjects ratings varied systematically with site and with level of exposure. As in Phase II, subjects gave higher ratings to symptoms in the eye, than to those in the nose and throat. There was no indication of intensification of symptoms for any parameter as the days of exposure progressed. For the eye, ANOVA statistics indicated level, and interaction of level by duration was significant. The ANOVA also revealed a smaller effect of level by day ($p<0.02$). For average ratings in the eye, nose, and throat see Table 4.

None of the subjects left the chamber prematurely during any of the four consecutive days.

Independent HED Analysis:

Responses to 100 ppb:

Five of 17 (29%) females and 7 of 15 (47%) males reported no ocular irritation during the first 30 minutes of a 60 minute exposure. For the second half of the 60 minute exposure (31-60 minutes), 4 females (23%) and the same 7 males as for the first 30 minutes reported no ocular irritation.

Scores of severe “3" ocular irritation were only sporadic during the first 30 minutes of exposure in both female (2; 12%) and male (4; 27%) subjects. Severe was defined as the symptom is hard to tolerate and can interfere with activities of daily living or sleeping. One female indicated sporadic severe scores at 7 separate time points during 2 days of exposure. Another female gave severe scores for 4 minutes during one day of 4 consecutive days. Four male subjects gave a severe score once or twice during the first 30 minute session.

A more consistent response in severity was observed in the second half of the exposure period (31-60 minutes). A total of 3 females (18%) and 4 males (27%) reported severe ocular irritation. The first female reported severe ocular irritation during 2 of 4 consecutive days. The second female subject rated severe effects from 31 minutes to 39 minutes of one day. The third female subject rated severe effects from 42 minutes to essentially 60 minutes of exposure.

Within male subjects, one male sporadically rated severe eye irritation during 3 days. The second male subject rated severe eye irritation from 41 minutes to 49 minutes in one day of 4 days. The third male subject rated severe eye irritation from 48 minutes to 55 minutes one day and sporadically in another day. The fourth male sporadically rated severe eye irritation in 2 of the 4 days.

Scores of moderate “2" ocular irritation were sporadic during the first 30 minutes of exposure in both female and male subjects. Moderate eye irritation was defined as “symptom definite and bothersome, but tolerated”. Moderate eye irritation was reported by the same subjects that reported severe eye irritation and in an additional 4 females and 2 males.

A more consistent response in moderate ocular irritation was observed in the second half of the exposure period (31-60 minutes). All female subjects that reported moderate ocular irritation during the first 30 minutes of exposure also reported a more consistent score during the last 30 minutes of exposure. Two additional female subjects rated moderate eye irritation after the first 30 minutes of exposure. All male subjects that reported moderate ocular irritation during the first 30 minutes of exposure also reported a more consistent score during the last 30 minutes of exposure. Two male subjects also sporadically reported moderate eye irritation after the first 30 minutes of exposure.

Responses to 150 ppb:
Three of 17 (18%) females and 5 of 15 (33%) males reported no ocular irritation during the 60 minute repeated exposures.

In contrast, 4 (23%) female subjects gave consist scores for severe eye irritation early during the 60 minute exposure. Specifically, one female that reported severe eye irritation at 100 ppb also gave consistent eye irritation scores beginning at 9 minutes of exposure for 2 different days and at 14 minutes on a third day with severe scores persisting almost to the end of the 60 minutes. A second female subject that reported severe eye irritation during the second half of exposure at 100 ppb reported severe eye irritation beginning at 11, 15, and 18 minutes of separate days until 58 minutes of exposure. A third female gave consistent severe eye irritation ratings beginning at 9 and 11 minutes until 60 minutes of exposure. A fourth female subject did not report severe eye irritation at 100 ppb but reported severe eye irritation at 16 to 18 minutes of exposure until 58 minutes of exposure at 150 ppb. In addition, 5 females gave sporadic scores for eye irritation at 150 ppb.

The same 3 male subjects (20%) that reported severe eye irritation at 100 ppb also reported severe eye irritation at 150 ppb but earlier during exposure. Specifically, the first male subject reported severe effects at 8 minutes that sporadically lasted until 58 minutes. The second male subject reported as early as 13 minutes which lasted until 60 minutes. The third male subject reported severe effects as early as 15 minutes until 60 minutes. Comparing subject responses in phase III with phase II, 2 of the three males reporting severe eye irritation at 100 ppb and 150 ppb in phase III also reported high confidence to eye feel during some time point of phase II of the study (50 ppb to 150 ppb).

Scores of moderate eye irritation were more consistent at 150 ppb than observed at 100 ppb. Nine of 17 (53%) female subjects gave moderate scores of irritation beginning around 10 minutes of exposure. Likewise, 6 of 15 (40%) male subjects gave moderate scores of eye irritation beginning around 10 minutes.

Table 6. Group responses to chloropicrin (phase 3-chamber) at 1hr periods over 4 consecutive days

Exposure	Ocular Average Rating (all subjects)	Definition	Time for Recognition	Nasal Avg Rating	Time for Recognition	Throat Avg Rating	Time for Recognition
blank	0.1	No symptom	NA	0.1	5 minutes	0.1	5 minutes
100 ppb	0.5	Between no symptom and mild symptom	30 minutes	0.1	5 minutes	0.1	5 minutes
150 ppb	1	Mild; Symptom present, but minimal awareness, easily tolerated	20 minutes	0.2	5 minutes	0.1	5 minutes

Data taken from final study report pg 67 and Figure 20 (pg 69). NA = not available.

B. Daily Measurements (Pre- and Post-Exposure):
Assessments on the days of exposure (1 hr/day for 4 consecutive days) included measurements of pulmonary function, nasal and lung nitric oxide (nNO and eNO, respectively), and of nasal airflow, as well as ratings of signs and symptoms. Pre-exposure is the period before exposure and post-exposure refers to the one hour after exposure on the days of exposure.

Probabilities of both signs and symptoms were run to determine differences between pre-exposure and post-exposure measurements. The average probability ratings for nasal congestion were 0.19 pre-exposure and 0.23 post-exposure. The average probability rating for ocular irritation pre-exposure was 0.1 at each concentration compared to 0.1, 0.3, and 0.4 for post-exposure measurement to blank (air), 100 ppb, and 150 ppb. The average probability rating given for symptoms other than nasal congestion and eye irritation equaled 0.1 on the scale of 0 (None) to 3 (Severe). Again, only ocular irritation showed any association between incidence and level of exposure. Nine percent of subjects expressed a symptom of ocular irritation before exposure with 27% after 100 ppb and 35% after exposure to 150 ppb.

Cochran Q test
Computation of tallies of the number of times a rating post-exposure exceeded a rating pre-exposure indicated nasal congestion was not significant ($Q=0.75$) while eye irritation was significant ($Q=28.8$, $p<0.0001$). Nasal congestion and ocular erythema (redness) occurred more than the trivial frequency. However, the ocular irritation did not translate into more prominent redness.

C. Lower Respiratory Variables:
1. FVC (Forced Vital Capacity): ANOVA indicated a significant interaction of level by order ($p<0.05$), however, there was only a 3% variation in FVC. The interaction of level by order by day did not achieve significance. Sex was not significant in any of the relevant interactions.

2. FEV_1 (Forced Expiratory Volume), the second of three lower respiratory variables, averaged 93.6% before exposure and 93.7% after exposure. As with FVC, the variation spanned only 3% and did not achieve significance. Sex was not significant in any of the relevant interactions.

3. The third lower respiratory variable, concentration of nitric oxide (eNO), exhaled from the lungs, equaled 37.8 ppb before exposure and 39.2 ppb after exposure with no significance achieved. Again, sex was not significant in any of the relevant interactions.

D. Upper Respiratory Variables:
Nasal nitric oxide (nNO) sampled from the nose was significant for level by order with 399 ppb before exposure and 425 ppb after exposure ($p=0.012$). However, level by order by day was not significant. nNO increased 1% after exposure to blank, 10% after exposure to 100 ppb, and 8% after exposure to 150 ppb. Sex was not significant in any of the relevant interactions.

Inspiratory flow and expiratory flow equaled 450 and 415 mL/sec, respectively, before exposure and 435 and 406 mL/sec, respectively, after exposure. Chloropicrin exposure had a differential effect on flow. Level by order was nearly significant ($p=0.087$) with level by order by day not significant. Flow decreased 2% after exposure to blank and increased 2% after exposure to 100 ppb chloropicrin, however, flow decreased by 8% after exposure to 150 ppb. Sex was not significant in any of the relevant interactions.

E. Beginning vs. End of a 4 -day Cycle:
Neither the lower respiratory nor the upper respiratory variables showed any cumulative differential effect of the exposure to chloropicrin after 4 days. The cell types and cell numbers from the Rhinoprobe samples (nose or eye) were approximately the same at the end of a cycle as at the beginning. The results of the RQLQ questionnaire indicated the majority of subjects answered “not troubled at all” to almost all of the items. Nasal congestion was the only parameter that reached a level where more than one-half of the subjects gave a response above zero. Fifty-three percent of subjects reported a non-zero response to congestion after the 4 days of exposure to the blank vs. 41% and 34% after the exposures to 150 ppb and 100 ppb, respectively. The average ratings equaled 0.53, 0.34, and 0.41 for the blank, 100 ppb, and 150 ppb, respectively, where a rating of 1 signified “hardly troubled at all”. A minority of subjects reported symptoms in the eyes, three of those symptoms showed a differential effect of exposure, specifically, watery eyes, sore eyes, and swollen eyes. The Q test revealed significance for sore eyes only ($p<0.05$). The highest rating given after exposure to swollen eyes equaled 0.47.

Summary of Results for Phase III:
Chloropicrin at 100 ppb and 150 ppb had both perceptual and physiological effects. Ocular irritation was mild in the average person with the more sensitive individual rating severe eye irritation that was defined as “symptom hard to tolerate and can interfere with activities of daily living or sleeping”. Clinical examinations an hour after exposure indicated no chloropicrin-associated signs of the exposures, though at times some subjects rated residual ocular symptoms. The symptoms did not worsen during the four days of exposure, but dissipated between exposures and none existed the day after the final exposure of a cycle.
Concentration of nNO and flow in the nose changed in association with exposure to chloropicrin from pre- to post-exposure in one day. nNO increased despite the absence of nasal symptoms and did not persist to the following day nor became progressively greater as the week of exposures wore on. The absence of increase nNO on the final day along with the no increase in neutrophils suggested a recovery from inflammation or a possible non-inflammatory basis for the elevation. However, nasal congestion and engorgement may have lead to the changes in nNO and flow.

The LOAEL is 100 ppb, the lowest concentration tested, based on eye irritation, increased nasal nitric oxide (nNO), and differential effect on inspiratory and expiratory flow. A NOAEL was not established in Phase III.

VIII. SUMMARY OF THE 3 PHASES:

Summary table of chloropicrin odor detection and eye, nose, and throat irritation in healthy subjects.

Table 7. Summary of results for the three phases of exposure to chloropicrin

Exposure Concentration	Concentrations	Exposure Duration	NOAEL/Threshold	LOAEL/Threshold	Effect
Phase I	0, 356, 533, 800 or 1200 ppb	"sniff"	Study Report Analysis: median odor threshold: 700 ppb M=590 ppb and F=810 ppb; median eye irritation: 900 ppb; M=790 ppb and F=1010 ppb; No localization of the throat; >1200ppb. Independent HED Analysis: 50th percentile for Odor: 356 ppb; 50th percentile for Eye: >356 but < 533ppb		
Phase II	0, 50, 75, 100, or 150 ppb	20-30 minutes	One subject left at 75 ppb; 4 subjects left at 150 ppb	38% initially and consistently detected chloropicrin at 50 ppb (16 of 42)	ocular and nasal detection of sensitive individuals
Phase III	100 or 150 ppb	60 minutes/day for 4 consecutive days	Not Established <100 ppb	100 ppb	ocular irritation, increased nasal nitric oxide, differential inspiratory and expiratory flow, 30 minutes to steady state

data taken from MRID 46443801

At short "sniff" or momentary exposures, a majority of the individuals identifying the odor of chloropicrin did so initially at the lowest concentration (356 ppb) and consistently to the highest concentration (1200 ppb). The calculated median of those subjects detecting odor was 406 ppb. The median concentration for ocular irritation for momentary exposures was calculated at 242 ppb. However, ocular irritation was detected by some individuals at much lower concentrations but only during longer durations, namely 20 to 30 minutes. Results of this study indicate eye irritation may occur before odor detection for some individuals. However, the variability of the subject's responses to chloropicrin was large. Approximately 30% of subjects did not report eye feel from chloropicrin at the highest dose tested (150 ppb) for 30 to 60 minutes (Phase III). Individuals not detecting chloropicrin at the lower concentrations would not have an early indication of exposure to chloropicrin and unknowingly may remain in an exposure scenario. The individuals more sensitive to the irritating effects of chloropicrin in the eye might be more prone to leaving the area of the chloropicrin exposure. Therefore, there is a concern of the use of chloropicrin as an odorant for warning of other chemicals, since results from this study indicate eye irritation is detected at lower concentrations, albeit for 20-30 minutes, than odor.

Addendum 1

Statistical Analysis of Phase I and Phase II of the Chloropicrin Human Irritation Study. 2005. Carol Christensen and David Miller, Chemistry Exposure Branch, Health Effects Division.

Human Eye Irritation and Odor Threshold Study

The Chloropicrin Manufacturers Task Force (the Task Force) performed a non-guideline, special study to determine the sensitivity of participants to chloropicrin through both direct and indirect exposure. Phase I established the sensitivity of participants to the chemical through exposure applied directly to the organ of interest, *i.e.*, eye or nose, using specifically designed exposure equipment. Phase II ascertained the sensitivity of test participants to chloropicrin through indirect exposure in a full-body chamber. In Phase II, researchers investigated irritation of the eye, nose and throat through ambient exposure to chloropicrin. Phase III of the Chloropicrin Manufacturers Task Force human is not examined in this analysis. The Office of Pesticide Program's (OPP) Health Effects Division (HED) believes the Phase II dosing scenario is the exposure scenario that best approximates that which will be encountered by workers and bystanders and is the exposure scenario that is of most interest to OPP. HED performed a statistical analysis of the submitted raw data to determine the distribution of chloropicrin detection levels through eye irritation and odor sensation in Phase I and Phase II of the Chloropicrin Manufacturers Task Force's "Special Study: Human Eye Irritation and Odor Threshold Study."

Phase I

In Phase I, participants experienced brief (1-2 second) contact with chloropicrin through direct ocular and nasal exposure using specially made exposure devices. Each of the participants was asked to judge whether the chemical was present in approximately 30 (27-30) trials for each of 4 dose levels (356, 533, 800, 1200 ppb). Each trial consisted of three 'cones' or exposure devices, one of which contained chloropicrin. In each trial, participants recorded which exposure device contained chloropicrin based upon sensation or irritation to the nose (odor detection) and eye, respectively. There were 62 participants who completed the odor detection part and 63 participants who completed the eye irritation part of Phase I. These data provide the basis for the distribution describing chloropicrin detection in the sample.

To perform this task, HED recorded the number of positive detections (a participant's judgement that the chemical is present in the exposure device) as a proportion of the total number of trials and 'corrected' this proportion to reflect the proportion of positive detections required to consider an individual participant to have positively detected the chemical *beyond chance at each dose level*. An individual participant may correctly determine the presence of the chemical in the exposure device in some of the trials by chance or pure guess. By 'correcting' for participants' chance occurrence of a positive detection of the chemical, HED was able to generate a *distribution of detection levels* which reflects the true dose

levels at which participants are able to detect the chemical through eye irritation or odor detection.

HED utilized a scoring method similar to that used in some multiple choice exam to remove the effect of chance or guessing in the recording of total number of positive detections. The following algorithm is used to determine the score for each study participant:

Score=total # positive detections - 0.5 (total # trials - total # positive detections)

Given an individual participant's score, that participant is classified as either a 'true detector' or a 'non-detector' (1 or 0, respectively) for each of the four dose levels using the criterion:

Detect=1 if Score>0.33(# detects)
Detect=0 if Score≠0.33(# detects)

In Phase I, participants were also asked to provide a confidence score at each trial. Participants rated their assurance on a scale of 1-5 reflecting how confident they were that the chloropicrin was or was not in the exposure device. This information was not incorporated or otherwise considered in the analysis.

The next step is to determine the dose level at which each participant in the study *initially and continuously* detected the chemical. To do this, HED placed a rule on the data: participants were not allowed to be scored as 'true detectors' at one dose level and 'non-detectors' at a higher dose level. There is no biological reason to assume some participants would be able to detect chloropicrin at a low dose level but not be able to detect chloropicrin at a higher dose level. Therefore, some alterations were made to the data set to reflect the *amended* criterion:

Detect=1 if Score>0.33(# detects)
Detect=0 if Score≠0.33(# detects)

Unless non-monotonic

The amended criterion ensures each participant was a monotonically increasing or constant chloropicrin non-detector or detector across the increasing dose levels of Phase I (345, 533, 800, and 1200 ppb). The tables below enumerate the total number of participants who initially and continuously detected the chemical at each of the four dose levels in both the odor detection and eye irritation parts of Phase I. The numbers of adjustments made given the amended criterion noted above are also shown in the following tables.

Table 1: Chloropicrin Detection by Odor Sensation (Phase I)

Dose Level (ppb)	# Participants[1]	Total	Percent Detect	Adjusted[2]
356	38	54	70	0
533	7	54	13	0
800	6	54	11	2
1200	3	54	6	1
Total	*54*	*--*	*100*	*3*
1200+ ppb (no detect at HDT)	8[3]	62	13	4

[1]Number of participants who initially and continuously detect the chemical at each dose level.
[2]Participants must consistently detect chloropicrin; adjusted in 7 out of 62 participants.
[3]There are 4 true non-detectors; 4 non-detectors by amended criterion.

Using a simple tally method, the 50th percentile of the distribution of 'true detectors' is the 27th observation of the 54 persons detecting the chemical, or 356 ppb[5]. Participants who could not detect chloropicrin at any dose tested (even the highest dose) were omitted from the analysis (n=8).

[5]Using logistic regression to model the probability of positive detection, key percentiles of the distribution are: the 10th percentile of detection is 215.84 ppb; the median is 405.8 ppb; and the 90th percentile is 763.7 ppb based upon the equation-- ln(odds of detect)=20.9 - 3.48(log of dose). Because the median derived from the logistic operation is based upon the calculated slope of the line, it is slightly different from the median result from the simple tally method.

Table 2: Chloropicrin Detection by Eye Irritation (Phase I)

Dose Level (ppb)	# Participants[1]	Total	Percent Detect	Adjusted[2]
356	19	56	34	7
533	19	56	34	0
800	13	56	23	0
1200	5	56	9	0
Total	*56*	--	*100*	*7*
1200+ ppb (no detect at HDT)	7[3]	63	11	5

[1]Number of participants who initially and continuously detect the chemical at each dose level.
[2]Participants must consistently detect chloropicrin; adjusted in 12 cases.
[3]There are 2 true non-detectors; 5 non-detectors by amended criterion.

Using the simple tally approach, the median level of detection by quick eye exposure is between 356 ppb and 533 ppb[6]. Participants who could not detect chloropicrin at any dose tested (even the highest dose) were omitted form the analysis (n=7).

Phase II

During Phase II of the study, participants entered a whole-body (ambient) exposure chamber and were exposure to chloropicrin over a 20- or 30-minute exposure interval. The four dose levels investigated in this phase of the chloropicrin human study were 50, 75, 100, 150 ppb. The Chloropicrin Task Force study directors investigated each dose level separately. For each dose level, participants assessed whether chloropicrin was present in the chamber using a 6-point scale. The 6-point scale reflected both the participants' judgement as to whether or not the chemical was present in the chamber as well as a level of confidence in the judgement. The scores 1, 2 and 3 indicate participants believed chloropicrin was not in the chamber with low, medium and high confidence respectively, and scores of 4, 5, or 6 indicate that chloropicrin was in the chamber with similar confidence ratings. Participants assessed whether the chemical was present in the chamber based on the level of irritation

[6]Using logistic regression key percentiles of the distribution by eye irritation are: 10th percentile is 37.56 ppb; the median is 242.5 ppb; and, the 90th percentile is 1565.5 ppb using the equation ln(odds of detect)=6.48 - 1.18(log of dose). Because the median derived from the logistic operation is based upon the calculated slope of the line, it is slightly different from the median result from the simple tally method.

to the eye, nose and throat at 30 seconds, 1-minute and each minute thereafter that participants remained in the whole-body chamber. For example, if an individual rates the presence of the chloropicrin in the chamber at minute 9 with a score of 5, that participant believes the chemical is present due to irritation symptoms to eye, nose or throat with a moderate level of confidence at minute 9 of the 20 or 30 minute exposure period.

Figure 1 illustrates individual participant's eye irritation score over time by dose level. The figure demonstrates that as both exposure time and dose level increases, the median irritation score increases. The figure also shows that even at the lowest dose and early in the exposure period, there are some individuals who detect the chemical with moderate to high confidence. Also, as discussed below, even at the highest dose tested (150 ppb) there are some individuals who cannot detect the chemical.

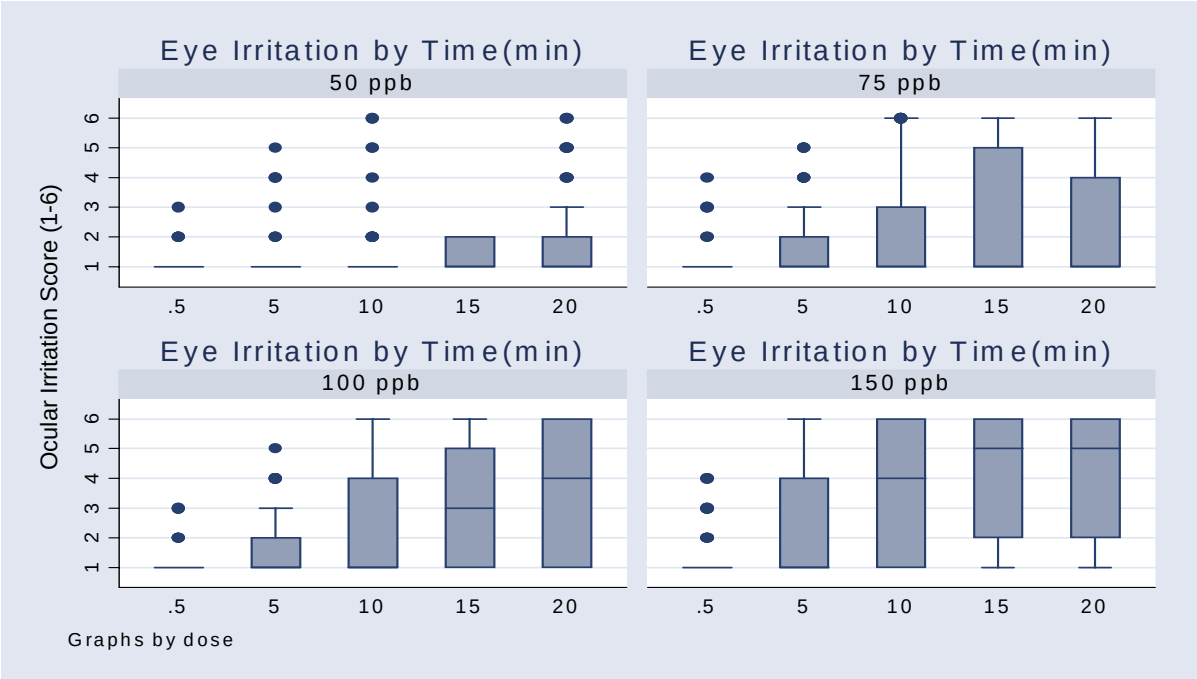

Figure 1: Participant's Eye Irritation Score over Exposure Period by Dose Level

HED classified each participant as to whether or not the participant could detect the chemical during each of the exposure periods. In this analysis, HED defined a 'true detector' as a participant who detected the chemical in the exposure chamber with an irritation confidence level of 5 or 6 in any 2 or more exposure minutes during the 20 or 30 minute exposure period. Therefore, participants who gave confidence scores of 1-4 throughout the time period in the chamber were considered non-detectors and participants who gave confidence scores of 5-6 at least 2 time points in the exposure interval were considered detectors.

Binary detection indicators (yes/no) were combined by participant across dose levels. Similar to the Phase I analysis, HED determined the level at which

each participant *initially and consistently* detected chloropicrin. In Phase II, a significant proportion of participants could not detect chloropicrin at any dose level. For example, using eye irritation as a marker of detection of the chemical, 20 or the 62 participants could not detect the chemical. These participants were omitted from the analysis. Forty-six and 48 participants among 62 could not detect the chemical by nasal or throat irritation symptoms, respectively. Therefore, this analysis was only carried out for the eye irritation data collected in Phase II of the study.

Table 3: Phase II: Chloropicrin Detection by Eye Irritation through Ambient Exposure

Dose Level (ppb)	**# Participants[1]**	**Total**	**Percent Detect**	**Adjusted[2]**
50	16	42	38	2
75	5	42	12	2
100	11	42	26	0
150	10	42	24	1
Total	*42*	--	*100*	*5*
150+ ppb (no detect at HDT)	20	62	32	0

[1]Number of participants who initially and continuously detect the chemical at each dose level.
[2]Participants must consistently detect chloropicrin; adjusted in 5 cases.

For participants who rated their eye irritation level in Phase II at 5 or 6 for at least 2 intervals in the exposure period, 16 of 42 (62-20=42) or 38% initially and consistently detected chloropicrin at 50 ppb. Simple tally methods result in a median detection level of 75 ppb. Logistic regression methods were attempted for this data set, however, model fit was poor and estimates of the distribution of the log odds of detection were not made.