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OFFICE OF
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MEMORANDUM

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SUBJECT: **Human Studies Review Board:** Weight of Evidence Discussion for Acrolein

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This document describes the scientific support for deriving a point of departure for acrolein from a human inhalation study (MRID 47060601). This point of departure is applicable for acute inhalation exposure risk assessment.

1. Background and Introduction:

Acrolein, propylene aldehyde: CH_2=HC-CHO is a clear or yellow liquid with a burnt, sweet, pungent odor with an odor threshold of 0.25 ppm. It is readily soluble in water (212 g/L). It boils at 52.6 ^{o}C and has vapor pressure of 274 mm Hg at 25 ^{o}C. Acrolein is a reactive aldehyde primarily used as an intermediate in chemical manufacturing (acrylic acid, acrylates, DL-methionine) and as a biocide in agricultural and industrial water supply systems. Acrolein is unstable and polymerizes (especially under light or in the presence of alkali or strong acid) to form diacryl, a plastic solid (Merck Index, 1966). Acrolein can be formed in burning tobacco, wood, plastics, gasoline and diesel fuel, paraffin wax, and in the heating of animal and vegetable fats and oils at high temperatures. It is also found naturally in the body in very small amounts as a product of lipid oxidation and the metabolism of α-hydroxyamino acids.

2. Hazard Characterization and Database Summary

The toxicological evaluation of acrolein is available in the extensive reviews prepared by the Agency for Toxic Substances and Disease Registry (ATSDR, 2005) (http://www.atsdr.cdc.gov/toxprofiles/tp124.pdf; http://www.atsdr.cdc.gov/toxprofiles/tp124.html) and the EPA Integrated Risk Information System (IRIS 2003) http://www.epa.gov/IRIS/subst/0364.htm; Toxicological Review of Acrolein (PDF). The information below is reproduced from these reviews.

> "Acrolein is toxic by inhalation, oral, and dermal exposures (toxicity category I for all routes). It is a potent irritant to the mucous membranes. As such, its toxicity is exerted at the point of contact with tissues. Signs and symptoms resulting from inhalation exposure to airborne acrolein may include irritation of the nose, throat and lungs, pulmonary edema, lung hemorrhage, and death. The nasal tissues appear to be the most sensitive target of inhalation exposure, with onset of noticeable irritation occurring in seconds (0.3 ppm). Higher airborne concentrations of acrolein (2– 5 ppm) result in increasingly severe manifestations of irritation over the entire respiratory tract. Oral acrolein exposure may result in gastrointestinal discomfort, vomiting, and stomach ulceration and/or hemorrhage. The stomach epithelium appears to be the most sensitive target for oral exposure (0.75 mg/kg). Higher concentrations of ingested acrolein have primarily resulted in increasingly severe irritation effects in the stomach (2 mg/kg and higher). Exposure to acrolein vapors or liquids may cause stinging of the eyes, lacrimation, and reddening, ulceration, or necrosis of the skin (10% acrolein solution). The eye appears to be the most sensitive target for exposure (0.3 ppm). Histological changes in respiratory and gastrointestinal epithelium have been observed from both inhalation and oral exposures, respectively. Changes in body and organ weights, hematology, and serum biochemistry, as well as developmental effects such as skeletal malformations and reduced weight of offspring,

have been observed in animals. Some of these effects are believed to be secondary effects of gastrointestinal and/or respiratory tract irritation (i.e., loss of appetite and weight loss due to gastrointestinal irritation).

Inhaled acrolein is retained primarily in the upper respiratory tract (Egle, 1972) because of its high solubility and reactivity. Draminski et al. (1983) identified a low level of acrolein derived conjugates in the urine of rats following oral dosing. Orally administered acrolein is excreted (as metabolites) in the urine, feces and as carbon dioxide. The main pathway of metabolism for acrolein is the addition of GSH to the activated double bond followed by conversion to mercapturic acid. A second pathway is that of epoxidation of the double bond followed by attack on the epoxide by glutathione. A third pathway is addition of water to acrolein to form 3-hydroxypropionaldehyde, which can be further metabolized and ultimately incorporated into normal metabolic pathways (Parent et al., 1998).

In vitro studies have shown acrolein to be weakly mutagenic. The evidence for the carcinogenicity of acrolein is equivocal, with a significant tumor incidence found in a single animal drinking water study. Another well-designed cancer bioassay in rats orally-gavaged at lower doses failed to detect significant increases in cancer incidence. The Department of Health and Human Services (DHHS) has not classified acrolein as to its carcinogenicity. The International Agency for Research on Cancer (IARC) (http://www.inchem.org/documents/iarc/vol63/acrolein.html) has determined that acrolein is not classifiable as to carcinogenicity in humans. The EPA in 1987 (Carcinogen Risk Assessment Validation Effort (CRAVE) classified acrolein as Group C–Possible Human Carcinogen, based on adrenal cortical adenomas; in female Fischer 344 rats.

Occupational exposure to acrolein may occur through inhalation and dermal contact. The half-life of acrolein in drinking water suggests some potential for water to be a source of exposure to humans. Howard et al. (1991) estimated groundwater half-lives of 11 days under aerobic conditions and 14-56 days under anaerobic conditions. However, limited studies indicate that it has rarely been detected in drinking or well water (Glaze et al., 1989; Staples et al., 1985), and the short halflives of acrolein in surface waters make long range aquatic transport unlikely (CICAD, 2002).

Exposure of the general population occurs primarily through atmospheric contact (HSDB, 2003). EPA reported mean ambient acrolein concentrations of 14.3 $\mu g/m^3$ (6.2 ppb), ranging from 8.2 to 24.6 $\mu g/m^3$ (3.6 to 10.7 ppb), for two urban locations based upon data from 1961 to 1980 (U.S. EPA, 1993). Acrolein has been detected in exhaust gases from both gasoline engines (0.05-27.7 mg/m^3) and diesel engines (0.12-0.21 mg/m^3) (IARC, 1995).

Concentrations in indoor air may exceed outdoor levels 2- to 20-fold times (Environment Canada, 2000). Levels between 2.3 and 275 μg/m^3 have been reported in smoky indoor environments such as bars and restaurants (IARC, 1995). In residences where wood stoves were used, concentrations from 0.7-6.0 μg /m^3 have been reported (IARC, 1995). IARC (1995) noted that the acrolein concentrations in the smoke from various cigarettes ranged from 3-220 μg/cigarette. Levels as high as 463-684 μg/cigarette were reported (Kuwata et al., 1979). Jones et al. (1999) reported concentrations of acrolein in mainstream smoke ranging from 10 – 140 μg per cigarette, and estimated concentrations in side stream smoke in the range of 100 – 1700 μg per cigarette (IRIS 2003)"

A. Animal Data

There are a number of published studies investigating the irritation and toxic effects of inhaled acrolein. The EPA IRIS 2003 acrolein report presented a comprehensive review of these various studies for the purpose of deriving a Reference Concentration dose RfC applicable to human risk assessment. The following are excerpts for key animal studies from the IRIS report.

Feron et al. (1978) Study

"Feron et al. (1978) exposed four equal groups, each consisting of 20 Syrian golden hamsters, 12 Wistar rats, and 4 Dutch rabbits (equal numbers of each sex) to 0, 0.4, 1.4, and 4.9 ppm (0, 0.9, 3.2, and 11 mg/m^3) acrolein, 6 hr/day, 5 days/week for 13 weeks in whole-body exposure chambers. Duration-adjusted values are 0, 0.07, 0.25, and 0.9 ppm (0, 0.16, 0.57, and 2.0 mg/m^3). Histopathology was performed on all major organs/tissues, including three transverse sections of the nasal cavity. Of the three species, rats were the most sensitive to the effects of acrolein. Mortality (6/24 rats) occurred in the 4.9 ppm (11 mg/m^3) group and animals kept their eyes closed. No adverse clinical observations were reported for the other concentration groups. Incidence data were not reported, but histopathological changes in the nasal cavity, lung, larynx, and trachea were graded as slightly, moderately, or severely affected. Hematological parameters were unaffected by acrolein in rats. Body weight gain was significantly inhibited at the high dose ($p<0.001$) in rats and less so at the intermediate concentration ($p<0.05$), but food consumption appeared to be decreased in these groups as well. No other deaths considered to be treatment-related were reported in any of the species or exposure groups. Histopathologic changes described as "slightly affected" were found in the nasal cavity of 1 of 12 rats exposed to 0.4 ppm (0.9 mg/m^3). Severity increased at the higher levels of exposure. No nasal lesions were reported in rabbits or hamsters at 0.4 ppm

(0.9 mg/m^3). The severity of nasal lesions was concentration-related in all 3 species, most clearly so in the rat. In the 4.9 ppm (11 mg/m^3) groups of all 3 species, slightly to markedly increased lesions were reported in the nasal cavity and trachea; moderate to marked effects were seen in the bronchi and lungs of rats and rabbits (but not hamsters). Based on the apparent concentration-related increase in severity of nasal lesions (i.e., slightly to severely affected), IRIS considered 0.4 ppm (0.9 mg/m^3) as a minimal LOAEL (i.e., an exposure level close to the expected NOAEL). Even though only 1/12 rats at this concentration demonstrated minimal metaplastic and inflammatory changes, these effects were consistent with the pathology demonstrated at the higher concentrations in which severity was increased. The duration-adjusted LOAEL is 0.4 ppm (0.9 mg/m^3) x 6/24 x 5/7 = 0.07 ppm (0.16 mg/m^3).

Additional evidence in support of a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) is provided by the studies of Kutzman and colleagues (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986) and Cassee et al. (1996b). Kutzman and colleagues exposed male Fischer 344 rats (50/group) via inhalation to acrolein at 0, 0.4, 1.4, or 4.0 ppm (0, 0.9, 3.2 or 9.2 mg/m^3) 6 hr/day, 5 days/week for 62 exposure days (consecutive weekdays, except for weekends, for 12.4 calendar weeks). When rats were evaluated on the 6th day postexposure, some evidence of functional deficits was found at 0.4 ppm (0.9 mg/m^3) and more substantial damage at the highest concentration (4 ppm; 9.2 mg/m^3). The Cassee et al. (1996b) 3-day nose only study in the rat reported slight nasal effects at lower concentrations (0.25 ppm; 0.6 mg/m^3) than in the Feron et al (1978) whole-body inhalation study.

Additional support for acrolein's respiratory effects and association with increased mortality is provided by Kutzman et al. (1984). Female Dahl rats (which are derived from the Sprague-Dawley rat) that have been selected for either susceptibility (DS) or resistance (DR) to salt-induced hypertension were exposed to filtered air at 0.4, 1.4, and 4.0 ppm (0.9, 3.2 and 9.2 mg/m^3) acrolein. Ten DS and 10 DR rats/group were exposed 6 hr/day, 5 days/week for 61-63 days (consecutive weekdays, except for weekends, for 12.4 calender weeks). Animals were necropsied one week after final exposure or 13.3 weeks after the first exposure. All of the DS rats exposed to 4.0 ppm (9.2 mg/m^3) acrolein died within the first 11 days of exposure, while 60% of the DR animals survived to the end of exposure. Dose response increases in the severity of epithelial lesions occurred in both species with the DS rats being more sensitive, and demonstrating a different pathological response at the high-dose".

B. Human Data

1. MRID 47060601. Weber-Tschopp A, Fischer T, Gierer R, Grandjean E (1977) Experimentalle Reizwirkungen von akrolein auf den menschen (Experimentally Induced Irritating Effects of Acrolein on Man). Int Arch Occup Environ Health 40:117-130. (German) (Institute for Hygiene and Occupational Physiology, Swiss Federal engineering College, Zürich, Switzerland)

This study provides the most comprehensive description of acute effects in humans. Healthy male and female college student volunteers were exposed to acrolein in a 30 m^3 chamber at an 0.1 hourly air exchange rate in 3 trials:

(1) A continuous exposure at constantly increasing acrolein concentrations,
(2) Discontinuous short exposures to successively increasing concentrations, and
(3) Constant concentration for one hour.

Acrolein was injected with a micro liter syringe, vaporized and blown into the test chamber via a carrier gas stream. Acrolein concentration in the test chamber was quantitatively determined and results were reproducible.

In the first experiment, 31 male and 22 female students in groups of three participated. One trial with acrolein and one control trial under identical conditions but without acrolein were performed with each subject. Students were exposed to increasing acrolein concentration from 0 to 0.6 ppm in the first 35 minutes and to a constant 0.6 ppm concentration in the last 5 minutes. The subjects had to fill out a questionnaire every 5 minutes. The questions were: Is air quality good? Acceptable or bad? And do you have a desire to leave the chamber? After that, two subjects in each group were immediately compared for eye blinking frequency. With the third subject the breathing frequency during the entire exposure was measured. Eye irritation was significantly higher ($p<0.01$) than controls at 0.09 ppm and above. Nasal irritation was significantly higher ($p<0.01$) than controls beginning at 0.26 ppm. Throat irritation was experienced at 0.43 ppm and above. Eye blinking rate was experienced at 0.26 ppm and above ($p<0.01$). Respiration rate decreased by 25% ($p<0.01$) at 0.6 ppm concentration.

In the discontinuous short exposure experiment there were 42 students (17 males and 25 females). The subjects in groups of 4 were each exposed 5 times for 1 ½ minutes to variously high acrolein concentrations (0, 0.15, 0.3, 0.45, and 0.6 ppm). After a minute of exposure, they were given the questionnaire form to fill. Between each exposure they were allowed to recuperate in a clean room for 8 minutes. The same controls from the first experiment were used. Eye and nasal irritation was significantly higher ($p<0.05$) than controls beginning at 0.3 ppm and 0.06 ppm, respectively. Throat irritation was not reported.

In the constant one hour exposure duration, 46 students in groups of threes (21 males and 25 females) were exposed to 0.3 ppm acrolein concentration for 60 minutes. Measurements of eye blinking frequency, breathing frequency and subjective symptoms

of irritation were taken at the beginning of exposure and during exposure. Eye, nose and throat irritation reached a plateau after 20-30 minutes of exposure, while eye blinking frequency plateaued after 10 minutes. Respiratory rate decreased 20% after 40 minutes exposure ($p<0.01$). The severity of the annoyance significantly increased almost immediately after acrolein was introduced. Eye, Nose and throat irritation and eye blink frequency increased with increasing exposure duration. After 40 minutes the subjective irritation reached a constant intensity while eye blink frequency almost after 10 minutes reached a definite rate. Throat irritation, which was insignificant in the other exposures, reached significance after only 10 minutes at this long exposure. There was a significant individual correlation (p between <0.05 and <0.01) between eye blink frequency and the subjective eye irritation. Every person with a sharp increase in eye blink frequency also had a sharp increase of eye irritation.

The volunteers were asked about the air quality during the exposure if it was good, bad or for the desire to leave the chamber and the degree of irritation to the eyes, nose and throat. The effects to continuous exposure as well as discontinuous exposure increased with acrolein concentration. Some indication of adaptation to the irritating effects of acrolein was suggested by the study investigators. The eyes were more sensitive than the nose to the irritating effects of acrolein.

In the continuous exposure the irritation was significantly greater both in the eyes and nose than in the discontinuous short exposures. Throat irritation in both experiments was not as sensitive a criterion: in continuous exposure it increased significantly through 0.43 ppm, in discontinuous exposure it showed no change.

The eye blink frequency of 34 subjects in the continuous trial was a function of the acrolein concentration. It increased from 0.17 ppm to 0.26 ppm ($p<0.01$) and it doubled at about 0.3 ppm.

The breathing frequency of 19 subjects in the continuous exposure trial decreased slightly with increasing acrolein concentration. This decrease was statistically significant at 0.6 ppm ($p<0.05$). At this concentration the decrease in breathing frequency reached 4 breaths per minute - a decrease corresponding to about 25%.

An increase in irregular breathing frequency in 11/19 subjects compared to controls was observed, very soon after the addition of acrolein but mostly in the second half or last third of the exposure time. Nearly half of the subjects displayed more or less pronounced tendency to lengthen the expiration cycle or more rarely the inspiration cycle holding the breath toward the end of the acrolein exposure.

Based on the results of this investigation, it was concluded that the threshold for the effects measured are:

Eye irritation	0.09 ppm
Nasal irritation	0.15 ppm
Eye blink frequency	0.26 ppm

Breathing frequency	0.30 ppm
Throat irritation	0.30 ppm

C. Point of Departure and Uncertainty Factor(s)

The Weber-Tschopp *et al*, 1977 human study provides useful information in establishing a PoD for the acute inhalation bystander exposure scenario. This human study employed a large number of subjects (53, 42, and 46 individuals in the three experiments, respectively) who elicited subjective complaints. The acrolein concentrations in the inhalation exposure chamber were documented analytically and were within ± 10% of target concentrations. However, the study did not use blind controls. The subjects used in this study expected the acrolein exposures and it may have biased their subjective reactions. In spite of its limitations, the study demonstrated that subjective eye irritation was the most sensitive indicator for the acute acrolein exposure in humans with a threshold effect of 0.09 ppm (0.2 mg/m^3). Protection of the eyes from the irritating effects of acrolein will protect against other respiratory effects of nasal and throat irritation and breathing effects which occurred at slightly higher thresholds. As stated in the ATSDR (2005) report, page 60: "The ocular effects observed in experimental animals are qualitatively similar to those described in humans. Concentrations of acrolein higher than 1.0 ppm (1.8–3.7 ppm) caused eye irritation in dogs and monkeys as evidenced by lacrimation and closing of the eyes, but guinea pigs and rats appeared to be less sensitive, since 3.7 ppm had no noticeable effect (Lyon et al. 1970). No histological evaluation of the eye was conducted, but other reports indicate that ocular discharge was commonly seen (Murphy et al. 1964; Skog 1950)". An additional support for the Weber-Tschopp, 1977 study findings is that "nasal irritation in humans has been observed at levels similar to those seen in animals" as stated in the ATSDR 2005 report, page 14. Based on the nose and throat irritation and a decrease in respiratory rate in humans exposed to acrolein, ATSDR derived an acute-duration MRL of 0.003 ppm calculated from the LOAEL of 0.3 ppm in the Weber-Tschopp et al. 1977 study.

Therefore, a threshold of 0.09 ppm (0.2 mg/m^3) for the irritation effects of acrolein in humans is an appropriate endpoint for risk assessment of short term inhalation exposures. Because a human study is being used for the acute inhalation exposure scenario for acrolein, an interspecies uncertainty factor is not necessary. To account for the individual variability, an intraspecies uncertainty factor of 10X is applied to the selected LOAEL of 0.09 ppm (0.2 mg/m^3). Because a minimal LOAEL threshold effect is used, a 3X uncertainty factor is sufficient. A total of 30X uncertainty factor is applied to the endpoint of 0.2 mg/m^3 yielding an acute concentration of concern of 0.007 mg/m^3

IRIS derived an inhalation reference concentration (RfC) for the chronic exposures to acrolein based on the Feron *et al* (1978). A "minimal" LOAEL of 0.4 ppm (0.9 mg/m^3) based on nasal effects in rats was derived from this study. IRIS adjusted the LOAEL from the dosing regimen of 0.9 mg/ m^3 for 6 hr/day, 5 days/week for 13 weeks to a continuous exposure for an adjusted LOAEL of 0.16 mg/m^3. A dosimetically LOAEL adjusted ($LOAEL_{HEC}$) to a human equivalent concentration (HEC) of 0.02 mg/m^3 was then derived. The $LOAEL_{HEC}$ was used as the point of departure for calculating the RfC.

A total uncertainty factor of 1,000 was applied to this point of departure: 3 ($10^{½}$ for extrapolation from animal to humans (UF_A), 10 for intrahuman variability (UF_H), 10 for subchronic to chronic duration (UF_S), and 3 ($10^{½}$) for use of a minimal LOAEL (UF_L) to yield and RfC of 0.00002 mg/m^3). This might be appropriate for assessing risk for chronic exposure to acrolein but not appropriate for the risk assessment for acute exposures to acrolein.

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