MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

TO: File for n-Nonane (CAS# 111-84-2)

FROM: Doreen Lehner, Toxics Unit, Air Quality Division

DATE: February 2, 2017

SUBJECT: n-Nonane (CAS# 111-84-2) ITSL change in the averaging time from 24 hours to annual

The initial threshold screening level (ITSL) for n-nonane is 550 µg/m^3 based on an annual averaging time. The ITSL was originally established on 4/12/2004 and was based on a Carpenter et al, (1978) 63-day vapor inhalation study in male rats. Twenty-five rats per group were exposed to 0, 2.5, 5, or 10 mg/L. The effects of exposure to n-nonane included statistically significant lower mean body weights, salivation and lacrimation, loss of coordination and fine tremors. 2 rats died at the highest exposure level (10 mg/L). The no-observed-adverse-effect-level (NOAEL) was determined to be 3.1 mg/L. As the key study used to derive the ITSL is a 63-day inhalation study, the averaging time is being changed from 24 hours to annual.

References:

Act 451 of 1994, Natural Resources and Environmental Protection Act and Air Pollution Control Rules, Michigan Department of Environmental Quality.

Carpenter CP, Geary DL, myers RC, Nachreiner DJ, Sullivan LJ, and King JM. 1978. Petroleum hydrocarbon toxicity studies XVII. Animal Responses to n-nonane vapor. Toxicology and Applied Pharmacology 44:53-61.

MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

April 12, 2004

TO: File for n-Nonane (CAS No. 111-84-2)

FROM: Maggie Sadoff/ Mike Depa

SUBJECT: Derivation of Screening Level

The initial threshold screening level (ITSL) for n-nonane is 550 ug/m3 with a 24-hour averaging time.

BACKGROUND

A search of the literature and the following databases was performed for information regarding n-nonane: American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values, National Institute for Occupational Safety and Health (NIOSH) Pocket Guide to Hazardous Chemicals, Integrated Risk Information System (IRIS), NIOSH's Registry of Toxic Effects of Chemical Substances (RTECS), Environmental Protection Bureau Library, International Agency for Research on Cancer (IARC) Monographs, CAS Online (1967 to December 2003), Hazardous Substance Data Bank (HSDB), National Library of Medicine/Toxline, Health Effects Assessment Summary Tables (HEAST), and National Toxicology Program (NTP) Study Database.

Many of the references with adequate toxic endpoints in animal studies were for mixtures of the aliphatic fractions of fuels and solvents (e.g. C6-C12) which also contained n-nonane. Because the exposures in these studies were to mixtures of hydrocarbons rather than to nonane alone or to nonane as a predominant component of a mixture, data from these studies could not be used to establish a screening level for n-nonane. Similarly, human data were limited to reports of occupational exposures to complex mixtures of paints and other solvents (e.g. white spirits, jet fuel) and/or evaluation of internal doses (usually by blood or expired air samples). No toxic endpoints were given in the occupational studies. However, a report prepared by the Total Petroleum Hydrocarbon Criteria Working Group (1997) stated that the US Air Force was generating oral and inhalation data specifically for n-nonane. To date, this data remains unpublished.

N-nonane is a liquid paraffin hydrocarbon found in fuels, paint, and other solvents. The vapor pressure is 4.45mmHg at 25C (HSDB). In high doses n-nonane is a central nervous system (CNS) depressant. In the low doses typically seen in occupational exposures, it is primarily a skin and mucous membrane irritant. The ACGIH has set an 8-hour threshold limit value-time weighted average (TLV-TWA) of 200ppm (1050mg/m3) for occupational exposure to n-nonane and its isomers. The TLV is based on n-nonane's comparative toxicity to octane and is intended

to minimize the primary acute effects of n-nonane including CNS depression, narcosis, chemical dermatitis, and respiratory irritation (ACGIH). The NIOSH Recommended Exposure Level (REL) for nonane is also 200pm (NIOSH Pocket Guide to Chemical Hazards).

Animal Toxicity Studies

An acute 4-hr LC50 value in rats was reported by Carpenter et al. (1978) to be an average of 3200ppm (range was 2700-4000ppm). Observed effects were progressive with dose and included lacrimation, salivation, loss of coordination, clonic/tonic convulsions, tremors and death. The same research group conducted a 63-day, 6hr/day, 5 day/wk subacute inhalation toxicity study. Twenty-five male Harlan-Wistar rats were used in each of 4 exposure groups. Metered concentrations were set at 10, 5, 2.5, and 0 mg/L of n-nonane vapor (corresponds to measured concentrations of 8.4, 3.1, 1.9 and 0 mg/L). Endpoints monitored prior to sacrifice included body weight change, blood and urine analysis. At sacrifice, tissues taken from rats for microscopic evaluation included adrenal, brain, pituitary, trachea, bifurcation of the trachea, thyroid, parathyroid, lung, heart, liver, kidney, spleen, stomach, duodenum, pancreas, ileum, jejunum, colon, skeletal muscle, sciatic nerve, and bone marrow impression smear.

Two rats in the 10mg/L group died during day one of exposure. The authors noted lung congestion and hemorrhage at necropsy. The remaining rats in this group exhibited early toxicity symptoms such as salivation, loss of coordination and fine tremors throughout the first 4 days of exposure. Salivation and lacrimation were noted in the 10mg/L group throughout the remainder of the study and only during the 6 hour exposure period. Two rats in the 2.5mg/L group died – one after the 46^{th} exposure and one after the 52^{nd} exposure. However, the authors concluded that these deaths were unrelated to treatment. No signs of distress were reported in any of the other animal groups during the study.

Rats in the 10mg/L group consistently exhibited statistically significant lower mean body weights and mean body weight changes as compared to control rats. There was only one statistically significant blood chemistry result after 4 weeks of exposure in the 10mg/L group: an elevated serum glutamic pyruvic transaminase level (a clinical indicator for liver or heart damage). This difference was thought to be a transient effect as it was not noted in subsequent weeks. Histopathologic examination of respiratory and extrarespiratory tissues was performed on three animals from each exposure group after days 19 and 38 and also on all animals at serial sacrifice at weeks 4, 8 and 13. Only common sporadic lesions were found that were deemed unrelated to treatment by the pathologist.

It should be noted that the authors inconsistently report the duration of the exposure period. In tables and in the text of the report the authors alternately refer to the study as a 63-day or 13-week study. Since a 90-day study with exposures on 5 out of 7 days yields 64 days over a 3 month time span, we assume that the study is indeed subchronic and therefore sufficient from which to derive a reference concentration.

The no-observed-adverse-effect-level (NOAEL) for repeated 63-day inhalation of n-nonane vapor by rats was determined to be 3.1mg/L (590ppm) in this study.

In another inhalation toxicity study by Nilsen et al. (1988), an LC50 of 4467 ± 189ppm was calculated for n-nonane. Male Sprague-Dawley rats were exposed to inhalation of n-nonane at concentrations of 5280, 4438, 3560, and 2414 ppm for one 8-hour period with a 14-day observation period. In the highest exposure group (5280ppm), behavioral symptoms appeared after 2 hours of exposure and mortality appeared after 4 hours (there were 9/10 deaths at

5280ppm; 4/10 deaths at 4438ppm; 1/10 deaths at 3560ppm; and 0/10 deaths at 2414ppm). Behavioral effects monitored were general activity, coordination difficulties, tremor and spasms. The length of time to appearance of mortality and specific behavioral symptoms increased with decreasing exposure concentrations. Sedative and narcosis effects (reported by others) were not observed. This was an unusual finding in that alkanes in this class are widely accepted to possess anesthetic properties. No additional deaths occurred during the post-exposure 14-day observation period at any concentration of n-nonane. All animals surviving the 8-hour exposure period recovered from their symptoms during the 14-day observation period. Recovery times were dose-dependent (full recovery times were 7, 5, 1, and 0.2 days for 5280, 4438, 3560, and 2414 ppm groups, respectively).

All rats exposed to n-nonane showed 10% body weight decreases as compared to control groups due to an initial lag in weight gain of exposed animals during the first three days of observation. There were no remarkable findings in gross pathology between groups (total body weights, organ weights, morphological alterations of heart or kidney). The four animals that died during exposure to n-nonane at 4438ppm were autopsied and showed dilatation of liver sinusoids. Three of those four showed slight fatty changes in liver cells as well. No significant changes in liver were found at autopsy in animals surviving the initial 8-hour exposure period. Of the four deaths at 4438ppm, three showed marked pulmonary edema at microscopic evaluation. Two of those three had increased lung weights that were twice that of controls. All animals exposed to 4438ppm n-nonane exhibited a blue discoloration of the skin during exposure, suggestive of peripheral cyanosis and cardiopulmonary insufficiency. CNS symptoms in animals that died during 4438ppm n-nonane exposure were seizures and opisthotonus (muscle spasms which contort the body).

No macroscopic abnormalities of the brain were noted in any of the animals. There were no microscopic pathological findings in the brains of the four rats that died during 4438ppm of n-nonane exposure. However, in rats exposed to 4438ppm n-nonane and surviving through the 14 day post-exposure observation period, extensive changes were noted during light microscopic evaluation of 200 cerebellar neurons. These changes included rarification of Purkinje cells and severely damaged neurons that appeared to be segmental rather than random. Results from morphometry of the cerebellum performed on surviving animals from this same group demonstrated a clear loss of Purkinje cells that could not be solely attributed to reduction in cell size.

It was noted that within the narrow range of n-nonane concentrations evaluated in this study (2414 to 5280ppm), a wide range of effects were produced ranging from slight behavioral effects to 90% lethality. This observation would seem to indicate a steep dose-response curve, at least in this range of exposures.

The most significant finding is the loss of Purkinje cells in the cerebellar cortex. It could not be ruled out that the pathological findings in liver and lung may have been secondary to circulatory collapse. The loss of Purkinje cells in surviving animals, coupled with the lack of clinical signs in these animals during the 14 day observation period, suggests the potential for chronic subclinical neurotoxicity with the brain as a target organ of n-nonane toxicity.

Below is a summary table of reported values for toxicity of n-nonane:

Value Type	Concentration	Animal Type (sex, if identified)	Duration	Source (Pub Date)
LC50	3200ppm (avg.) (Note: range = 2700 to 4000ppm)	Rat (m)	4 hrs	Carpenter et al., 1978
LC50	4467+ 189ppm	Rat (m)	8 hrs	Nilsen et al., 1988
LD50	218 mg/kg	Mouse	--	RTECS
NOAEL (inhalation)	590ppm (3.1mg/L)	Rat (m)	63 days	Carpenter et al., 1978
NOAEL (oral)	100mg/kg	Fischer 334 rats (f) & C57BL/6 mice (m)	90 days	Dodd et al., 2000, unpublished data presented at 2000 SOT meeting
RfD	1.017 mg/kg/day	Extrapolation from Carpenter's rat inhalation study data	---	Report prepared by Staats Creative Sciences for USAir Force, Department of Defense, 1994
RfD	0.6mg/kd/day (based on potency vs. n-hexane)	---	---	MADEP (Mass Dept of Env Protection

Mutagenicity

N-nonane is not reported to be a human or animal carcinogen. All Ames tests for mutagenicity were negative (Chemical Carcinogenesis Research Information System,CCRIS). N-nonane was tested for its induction and promotion potential and its effect on intercellular communication in primary Syrian hamster embryo cells (Rivedal et al., 1992). Results were negative for induction of morphological transformation on its own and also for enhancement of transformation frequency induced by benzo(a)pyrene.

Other Evidence

Kristiansen & Nielson (1988) determined an upper limit for sensory irritation in the workplace for inhaled n-nonane vapor to be 125 ppm (654 mg/m3), based on the chemical's ability to activate the sensory irritant receptor in mice (also known as the trigeminal reflex). These researchers also cited an earlier paper by Gemert & Nettenbreijer (1977) who reported an odor threshold for n-nonane in humans to be 11-21 ppm (58-110 mg/m3). These values are well above the derived screening level and therefore the ITSL is protective of sensory irritation effects.

Several absorption/distribution studies of n-nonane inhalation toxicity in rats reported an affinity for inhaled n-nonane to accumulate in brain and fat tissue and to be retained with prolonged exposure (see Zahlsen et al., 1990 & 1992; Lof et al., 1999; Lam et al.,1992). This finding may have clinical significance for humans and, in fact, n-nonane has been detected in samples of breast milk and expired air in epidemiological studies of human subjects with no history of occupational exposures (Krotoszynski & O'Neill, 1982).

Limited *in vitro* evidence of n-nonane toxicity involves its ability to produce oxidative stress in rat brain and human neutrophils (Myhre et al., 2000, 2001). This evidence may be pertinent to human health when one considers the suggestive evidence of the potential for n-nonane bioaccumulation in fat and brain tissue in the rat. A recent epidemiologic study was conducted on the reproductive endocrine effects of inhaled n-nonane (as a component of a complex mixture) in women with occupational fuel and solvent exposures (Reutman et al., 2002). Significant association was found between lower pre-ovulatory LH in the urine of healthy, reproductive-age women and higher internal doses of aliphatic hydrocarbons in exhaled breath.

Though the consequence and/or persistence of this observation was not reported, and the C6 to C11 alkanes were examined as a group, this finding warrants further study of n-nonane for potential reproductive effects with chronic, low-dose inhalation exposure.

CONCLUSION

The data on chronic neurotoxicity of n-nonane is currently limited as is the data on potential reproductive effects. It should also be noted that since there is a current occupational exposure limit (OEL) standard for n-nonane, according to Rule 232(1)c, an OEL derived ITSL would be 10,470 ug/m3, with an 8-hour averaging time. However, since the ACGIH-TLV for n-nonane is based on octane and not specifically on n-nonane toxicity, it was deemed inappropriate to use this OEL for the derivation of an ITSL.

The subacute inhalation study by Carpenter et al. (1978) is of sufficient quality from which to derive a reference concentration (RfC) for chronic inhalation exposure to nonane according to the EPA methodology (1994). The critical effects observed by Carpenter et al. include only remote, extrarespiratory effects (CNS effects). N-nonane is reported to have acute respiratory effects such as tissue irritation, but these effects were not reported in this study and typically only occur at relatively high exposure levels (i.e. acute occupational exposures).

By EPA criteria, nonane is classifiable as a Category 2 gas with limited solubility and potential for both respiratory and extrarespiratory effects. Since the formula for Category 2 gases contains an inappropriate term (see EPA memo dated April 1997), the default methodology for deriving an RfC for Category 2 gases is to use the Category 3 formula which is solely for extrarespiratory effects. This was deemed appropriate since the critical effects of chronic exposure to inhaled n-nonane are extrarespiratory in nature (CNS effects).

The no-observed-adverse-effect-level (NOAEL) for repeated 63-days of inhalation exposure to n-nonane vapor by rats was determined to be 3.1 mg/L (590ppm) in this study. Based on this value, an RfC was calculated utilizing EPA methodology for Category 3 gases as follows:

$$RfC = \frac{NOAEL_{[Human\ Equivalency\ Concentration\ -\ HEC]}}{\text{Uncertainty Factors (UF)}}$$

*UF = 10 for human to sensitive human
10 for animal to human
10 for subchronic to chronic

$$\text{So, } RfC = \frac{NOAEL_{[HEC]}}{1000}$$

*When using the EPA (1994) dosimetric adjustment factor (blood/gas partition coefficient), the default uncertainty factor for extrapolating from animal to human data is 3. However, a factor of 10 was deemed appropriate in this instance because there was no animal or human blood/gas partition coefficient data. A full factor of 10 for animal to human extrapolation was necessary to account for the difference in animal and human dosimetry. Furthermore, the authors do not report details of histopathological results but rather only state that results were unremarkable. Also, upper respiratory tract tissue changes such as inflammation of the nasal and/or sinus cavities were not evaluated.

An additional uncertainty factor of 3 for database deficiencies was considered since no studies were found that addressed neurobehavioral effects nor were two-generation reproductive studies performed. However, it is not AQD's current practice to apply that additional uncertainty factor.

$$NOAEL_{[HEC]} = NOAEL_{[Adjusted\text{-}ADJ]} \times \frac{(H_{b/g})A}{(H_{b/g})H}$$

(animal blood/gas partition coefficient) / (human blood/gas partition coefficient)

When blood/gas partition coefficients for a specific chemical in animals and humans are unknown, the default value is 1 so this term drops out of the equation leaving the $NOAEL_{[HEC]}$ equivalent to the $NOAEL_{[ADJ]}$.

$NOAEL_{[HEC]} = NOAEL_{[ADJ]}$ = Exposure Concentration (mg/m3) x # of hours/day (h/24hr) x # of days/week (days/7 days)

$NOAEL_{[HEC]}$ = 3100 mg/m3 x 6/24 x 5/7 = 554 mg/m3

$$RfC = \frac{NOAEL_{[HEC]}}{1000} = \frac{554\ mg/m3}{1000} = 0.554\ mg/m3$$

RfC = 0.554 mg/m3 x 1000 = 554 ug/m3 or ≈ 550 ug/m3.

The RfC was used to establish the ITSL, pursuant to Rule 232(1)(a). **The ITSL for n-nonane is 550 ug/m3 with a 24 hour averaging time.**

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