



October 16, 2017

VIA EMAIL

**(P65Public.Comments@oehha.ca.gov AND
OEHHA COMMENTS UPLOAD PORTAL)**

Ms. Michelle Ramirez
Office of Environmental Health Hazard Assessment
P.O. Box 4010, MS-12D
Sacramento, California 95812-4010

Re: n-Hexane Draft Hazard Identification Document

Dear Ms. Ramirez:

On behalf of the National Oilseed Processors Association (NOPA), I am submitting comments on the draft Proposition 65 n-Hexane Hazard Identification Document (HID) for consideration by the Developmental and Reproductive Toxicant Identification Committee (DARTIC) and the Office of Environmental Health Hazard Assessment (OEHHA). The Institute of Shortening and Edible Oils (ISEO) also joins in these comments. The technical comments, authored by Dr. Jay Murray, are attached to this cover letter.

Established in 1930, NOPA's mission is to assist the U.S. soybean, canola, flaxseed, sunflower seed and safflower seed processing industries to be the most competitive and efficient in the world by utilizing the combined expertise, knowledge and resources of its members to foster market- and science-based policies. NOPA represents 13 member companies who process over 1.8 billion bushels of oilseeds annually at 64 plants in 20 states, including 58 plants that process soybeans.

ISEO is a trade association representing the refiners of edible fats and oils in the U.S. ISEO member companies process over 20 billion pounds of edible fats and oils annually, which are used in baking and frying fats, salad and cooking oils, margarines and spreads, confectionary fats, and as ingredients in a wide variety of foods.

N-hexane has long been used as a solvent during extraction of edible oils from seed crops such as soybeans, corn, cottonseed and safflower seed. Such edible oils are then further processed and used, among other purposes, for manufacturing foods, cosmetics and other consumer products. For these purposes, n-hexane has certain beneficial attributes, including the preservation of color and avoidance of unwanted characteristics such as bitter flavors. Further, its

higher volatility, relative to these edible oils, renders n-hexane readily removable from the final oils during processing.

Available information shows that levels of n-hexane remaining in edible oils after processing are *de minimis*, and largely non-detectable. Nevertheless, the listing of n-hexane as a reproductive toxicant, in the absence of sound science clearly showing a connection between exposures to n-hexane and reproductive harm, will stigmatize this widely used solvent in edible oil applications. That clear connection between the substance and harm is necessary to meet the standard for listing, preserves the integrity of Proposition 65 and ultimately avoids the need for unnecessary warnings, a problem recognized by OEHHA as well as the community at large (*see, e.g.*, Editorial, “Warning: Too many warning signs are bad for your health,” *Los Angeles Times* (September 30, 2017), found at <http://www.latimes.com/opinion/editorials/la-ed-proposition-65-warning-coffee-20170930-story.html>).

As discussed in the attached comments, n-hexane does not meet the standard for listing as a reproductive toxicant under Proposition 65 – that is, n-hexane is not “clearly shown through scientifically valid testing according to generally accepted principles to cause ... reproductive toxicity.” Further, although it may be appropriate to evaluate the mechanism of purported harm in reviewing a proposed chemical listing, there is no basis for fully conflating a chemical proposed for listing with its Proposition 65-listed metabolites, as the draft HID appears to suggest here for n-hexane and its Proposition-65 listed metabolites, methyl n-butyl ketone (MnBK) and 2,5-hexanedione (2,5-HD).

In considering this issue, it is appropriate to turn to the words of the statute itself. Proposition 65’s warning requirement and discharge prohibition refer to “a chemical.” The listing provision also refers to “a chemical.” In terms of the operative provisions and the listing provision, Proposition 65 contemplates a distinct chemical, not a parent chemical and its metabolites.

Neither the statute nor the implementing regulations define “chemical.” In these circumstances, the legal rules of interpretation require turning to the commonly understood meaning of the undefined term. The American Heritage Dictionary defines “chemical” as a “substance with a *distinct* molecular composition that is produced by or used in a chemical process.”¹ This definition is consistent with the definition of the same term used in other statutes, such as the federal Toxic Substances Control Act: “any organic or inorganic substance of a *particular* molecular identity, including— (i) any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature, and (ii) any element or uncombined radical.”²

Critical to the definition of “chemical” is the concept of a *distinct* molecular composition. Here, n-hexane is *distinct* from MnBK and 2,5-HD in molecular composition. For that reason alone, it cannot be considered – either from a legal or scientific perspective – to be equivalent to MnBK or 2,5-HD. Thus, the DARTIC cannot assume that the listing of these metabolites automatically necessitates the listing of n-hexane. Such an assumption, in addition to

¹ American Heritage Dictionary, Fifth Ed. (2012) (emphasis added).

² 15 U.S.C. §2602 (emphasis added).

contravening Proposition 65 itself, would erode the scientific integrity that must be preserved in any hazard evaluation of a chemical.

The DARTIC must consider the proposed listing of n-hexane on its own terms, based on studies involving that chemical and not its metabolites. In this regard, an appellate court opinion is instructive. In *Consumer Cause, Inc. v Weider Nutrition Information, Inc.*,³ the appellate court analyzed whether a Proposition 65 warning was required for a dietary supplement that contained a chemical which was not itself listed, but which increased the levels of the body's testosterone, a listed chemical, when ingested. The court adopted the Attorney General's view, which is essentially this: what happens to a chemical inside the body should **not** be considered when evaluating the legal and factual question of whether a warning is required. The only point for evaluation was what chemical came into contact with the outside of the body. In that case, the chemical that came into contact with the body was not listed. Therefore, no warning was required. Although that case did not involve a listing decision, its approach nevertheless should be implemented here, for implicit in the court's Proposition 65 analysis was the concept that the chemicals at issue were **distinct** from one another – and one was listed while the other was not.

Thus, for the reasons set forth herein and in the attached technical comments, and consistent with the goal of fostering science-based policies, NOPA and ISEO urge the DARTIC to refrain from identifying n-hexane as a reproductive toxicant under Proposition 65.

Respectfully,

Grimaldi Law Offices

By:



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Counsel for National Oilseed Processors
Association

Attachment

³ (2001) 92 Cal.App.4th 363.

**Comments
of the
National Oilseed Processors Association
and
Institute of Shortening and Edible Oils
on the
Draft Hazard Identification Document (HID)
“Consideration of n-Hexane for Listing under
Proposition 65 as Known to Cause Reproductive
Toxicity”**

October 16, 2017

Submitted to:

Office of Environmental Health Hazard Assessment (OEHHA)

**Members of the Developmental and Reproductive Toxicity
Identification Committee (DARTIC)**

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I. Summary

The draft Hazard Identification Document (HID)¹ for n-hexane identifies no epidemiological studies and a number of animal studies that have evaluated the potential developmental toxicity (10 studies), female reproductive toxicity (4 studies), and male reproductive toxicity (7 studies) of n-hexane.² However, the quality and reliability of these studies vary, as noted in the details of the Experimental Parameters section of Tables 1-3 of the draft HID. Many of these studies have significant limitations and shortcomings, and fall short of meeting the Proposition 65 standard for listing chemicals. That standard is a stringent one: In order for the Developmental and Reproductive Toxicity Identification Committee (“DARTIC”) to recommend listing n-hexane, the chemical must be “clearly shown through scientifically valid testing according to generally accepted principles to cause” reproductive toxicity.

These comments on the draft HID can be summarized with the following points:

Human Evidence of Developmental or Reproductive Toxicity: “No studies in OEHHA’s literature search were identified regarding reproductive effects in humans after exposure to n-hexane.”³

Animal Evidence of Developmental Toxicity: The results of the animal studies do not support a conclusion that n-hexane is clearly shown to cause developmental toxicity. Table 1 of the draft HID identifies 10 studies on the developmental toxicity of n-hexane, including six inhalation developmental toxicity studies in rats, two developmental toxicity studies in mice (one inhalation and one oral), and two inhalation dominant lethal studies in male mice. In the developmental toxicity studies in rats and mice, n-hexane showed little or no evidence of developmental toxicity except at doses that produced overt maternal toxicity and even death. Two dominant lethal assays of inhaled n-hexane

¹ OEHHA (2017) Draft Hazard Identification Document, Consideration of n-Hexane for Listing under Proposition 65 as Known to Cause Reproductive Toxicity, September, 2017.

² There are actually only 17 studies in total due to the fact that some studies appear in more than one table.

³ *Id.*, p. 5.

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in male mice revealed no evidence of a dominant lethal effect. Some studies identified in the draft HID do not constitute “scientifically valid testing according to generally accepted principles.” The limitations of studies are identified in the Experimental Parameters section of Table 1. Examples of the limitations of certain studies include: unknown composition of the test material, inadequate group size, insufficient number of doses to evaluate a dose-response relationship, lack of detail with regard to both methods and results, and improper statistical analysis (e.g., using the fetus, and not the litter, as the statistical unit). In summary, the evidence falls short of meeting the statutory standard that must be met before n-hexane can be listed as a developmental toxicant.

Animal Evidence of Female Reproductive Toxicity: Table 2 of the draft HID identifies four studies of female reproductive toxicity involving exposure to n-hexane. All four of these studies were conducted by the same group of investigators at the Fujian Health College, Fujian, China between 2012-2015. Based on these four studies, n-hexane has not been clearly shown to cause female reproductive toxicity.

It is difficult to compare the results of these studies for consistency of results because the experimental design and endpoints evaluated differed from study to study. Even so, the studies’ shortcomings and deficiencies render them unacceptable for evaluation in this instance, because they do not represent “scientifically valid testing according to generally accepted principles.”

Systemic toxicity was assessed in only two studies. Symptoms in one study (Liu et al., 2012) included: “appeared to be quiet to different degrees,” decreased body weight, decreased activity, decreased depilation, decreased appetite, rhabdomyolysis, “ulcers in the abdominal area,” and one death. In the other study (Li et al., 2014), high dose pregnant rats “had mental symptoms: irritability and an attack tendency.” In the study exposing animals to the highest concentration (approximately 24,000 ppm) of n-hexane, systemic toxicity was not assessed at all (Liu et al., 2013).

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The two developmental toxicity studies (Li et al., 2014, 2015) identified in Table 2 of the draft HID used inadequate numbers of animals (5 or 6 pregnant rats/group) to assess any endpoints of developmental toxicity, including endpoints of female reproductive toxicity among the offspring. It appears that the fetus/pup was inappropriately used as the statistical unit. The evidence that n-hexane causes female reproductive toxicity is no more compelling than the evidence that methyl n-butyl ketone (MnBK) or 2,5-hexanedione (2,5-HD) causes female reproductive toxicity, and neither of these metabolites of n-hexane was listed as a female reproductive toxicant by the DARTIC.

Animal Evidence of Male Reproductive Toxicity: Table 3 of the draft HID identifies 7 studies providing data on the potential male reproductive toxicity of n-hexane. The evidence that n-hexane is clearly shown to cause male reproductive toxicity is insufficient to meet the stringent listing standard. n-Hexane is suspected of causing male reproductive toxicity because it is metabolized to MnBK and 2,5-HD, and both of these chemicals appear on the Proposition 65 list as male reproductive toxicants. However, the potential for n-hexane to produce reproductive toxicity is likely to depend on whether the internal dose (peak levels and area under the curve at the target site) of these metabolites is sufficient to cause male reproductive toxicity. In fact, there is a clear difference between n-hexane and 2,5-HD regarding the potential to cause male reproductive toxicity. Scientists at US EPA conducted a short-term screening assay for spermatotoxicity in male rats, and 2,5-HD was clearly positive, whereas n-hexane was negative.

Other studies of n-hexane demonstrate inconsistent evidence of male reproductive toxicity, which was observed at dose levels that produced serious toxicity. DeMartino et al (1987) showed adverse effects on spermatogenesis among rats exposed for 16 hr/day for up to six weeks to 5000 ppm n-hexane, which was associated with significant signs of toxicity, including neuropathy and severe weight loss serious enough to warrant premature sacrifice of some animals. In contrast, no increased incidence of spermatotoxicity was observed in the absence of systemic toxicity among mice exposed to up to 5000 ppm n-hexane for 20 hours/day for five consecutive days (Mast et al.,

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1988c). Nylen et al. (1989) found atrophic changes in the seminiferous tubules among rats exposed to 1000 ppm n-hexane for 18-21 hours/day for 28 or 61 days; however, all rats that exhibited testicular damage also demonstrated severe atrophy of the muscles of the hindlimbs and reduced body weight. Rats (n = 6 rats/group) exposed to 1000 ppm n-hexane for 4 hours/day for 6 days/week for 415 days found an increased incidence of Leydig cell hyperplasia; however, no changes in spermatogenesis were reported and fertility was not evaluated (Imai and Omoto, 1999).

Conclusion: In conclusion, the overall scientific evidence is insufficient to demonstrate that n-hexane has been clearly shown to cause developmental or reproductive toxicity.

II. Introduction

These comments are submitted on behalf of the National Oil Processors Association (NOPA) and the Institute of Shortening and Edible Oils (ISEO). They set forth the scientific and regulatory reasons that n-hexane does not meet the Proposition 65 criteria for listing: specifically, n-hexane has not “been clearly shown through scientifically valid testing according to generally accepted principles to cause ... reproductive toxicity.”⁴ The Office of Environmental Health Hazard Assessment (OEHHA) has provided you a draft Hazard Identification Document⁵ (HID) for n-hexane. This submission provides our comments to OEHHA and to the Proposition 65 Developmental and Reproductive Toxicity Identification Committee (DARTIC) on the draft HID.

Significantly, no authoritative body has formally identified n-hexane as causing reproductive toxicity. OEHHA is required to place on the Proposition 65 list any substance that the U.S. EPA, U.S. FDA, NTP, or NIOSH – all authoritative bodies under Proposition 65 – has formally identified as a reproductive or developmental toxicant. Because n-hexane cannot be listed on the basis of the views of any of these authoritative

⁴ Health and Safety Code § 25249.8(b).

⁵ OEHHA (2017) Draft Hazard Identification Document, Consideration of n-Hexane for Listing under Proposition 65 as Known to Cause Reproductive Toxicity, September, 2017.

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bodies, n-hexane has been placed before the DARTIC to address the question of whether n-hexane has “been clearly shown through scientifically valid testing according to generally accepted principles to cause . . . reproductive toxicity.” If the DARTIC were to identify n-hexane as a reproductive toxicant, we believe it would be the first scientific or regulatory body to do so.

III. Statutory Listing Standard and the Guidance Criteria

Proposition 65 imposes a stringent standard for listing a reproductive toxicant. The statute provides that:

“A chemical is known to the state to cause cancer or reproductive toxicity . . . if in the opinion of the state’s qualified experts it has been *clearly shown* through *scientifically valid testing* according to *generally accepted principles to cause* cancer or reproductive toxicity”⁶

Anything less does not permit listing under this statute.⁷

To support this stringent standard, the DARTIC has developed Guidance Criteria (DARTIC, 1993) that provide both general principles and specific factors for the Committee to

⁶ Health and Safety Code § 25249.8(b) (emphasis added).

⁷ This stringent standard does not exist in a vacuum. It is inextricably tied to the words and intent of the operative provisions of Proposition 65, i.e., the warning requirement and the discharge prohibition. The warning requirement requires businesses, under specified circumstances, to provide warnings for exposures “to a chemical known to the state to cause cancer or reproductive toxicity.” Health & Safety Code §25249.6. Similarly, the law prohibits businesses from discharging or releasing, under specified circumstances, “a chemical known to the state to cause cancer or reproductive toxicity.” Health & Safety Code §25249.5. Thus, the stringent standard for listing chemicals ensures that a chemical, to be encompassed by these two operative provisions, is actually *known* to cause the relevant harm as the law requires.

Meeting this statutory standard for listing is critical for executing the intent of the California citizens in voting for Proposition 65. The arguments in favor of Proposition 65 are unequivocal that the law’s purpose was to address chemicals that are “scientifically known” to cause cancer or reproductive harm. The arguments are also unequivocal that the law is to address chemicals “known to the state to cause cancer or reproductive disorders.” Only by imposing the stringent listing standard – i.e., that a chemical must be “clearly shown through scientifically valid testing according to generally accepted principles” to cause cancer or reproductive toxicity – can the intent of the people be advanced.

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weigh in assessing whether the scientific data on a particular chemical satisfy the “clearly shown” standard. The general principles include the following:

“Developmental, and female and male reproductive effects ***shall meet at least one of the following criteria*** for recommendation as known to the State to cause reproductive toxicity.

- (a) ***Sufficient evidence*** in humans. . . .
- (b) Limited evidence or suggestive evidence in humans, supported by sufficient experimental animal (mammalian) data
- (c) ***Sufficient evidence*** in experimental animals (mammals), such that extrapolation to humans is appropriate.”⁸

The Guidance Criteria identify specific factors for the Committee to consider in evaluating whether the available data on a particular compound qualify as “sufficient evidence in experimental animals.” Whether animal studies are “sufficient evidence” is based on the following:

- “(1) The ***experimental design***, including overall protocol and numbers of animals and ***presence of appropriate controls***.
- (2) The exposure, in terms of ***route of administration***, is ***relevant to expected human exposures***
- (3) Number of dose levels, so that the presence of a ***dose-response relationship*** can be evaluated
- (4) ***Consideration of maternal and systemic toxicity***.”⁹

As discussed further herein, most of the studies submitted to the DARTIC for evaluation do not meet the DARTIC Guidance Criteria or otherwise do not meet the standard for listing.

⁸ Guidance Criteria at 3.A.-C. (emphasis added).

⁹ Guidance Criteria at 3.C.(1) -(4) (emphasis added).

IV. The DARTIC Should Not Conflate n-Hexane with Certain Metabolites of n-Hexane

The draft HID includes information relating to the listing of methyl n-butyl ketone (“MnBK”) and 2,5-hexanedione (“2,5-HD”). While both of these chemicals are among the many metabolites of n-hexane, they are chemically distinct from n-hexane. The DARTIC should refrain from conflating n-hexane with certain of its metabolites and from listing n-hexane simply because certain of its metabolites have been listed.

The studies of n-hexane addressing male reproductive toxicity do not provide evidence that n-hexane is “clearly shown ... to cause” that harm. It is overly simplistic to assume that n-hexane causes male reproductive toxicity because some of its metabolites cause male reproductive toxicity. The potential for n-hexane to produce reproductive toxicity is likely to depend on whether the internal dose (peak levels and area under the curve at the target site) of these metabolites is sufficient to cause male reproductive toxicity.

In fact, there is a clear difference between n-hexane and 2,5-HD regarding the potential to cause male reproductive toxicity. For example, scientists at US EPA conducted a short-term screening assay for spermatotoxicity, and both n-hexane and 2,5-HD were evaluated in the same assay under the same conditions (Linder et al., 1992). The results were markedly different. For this test, n-hexane or 2,5-HD was given to male rats orally by gavage twice daily for a total daily dose of 20,000 mg/kg/day. Exposure to 2,5-HD produced substantial spermatotoxicity after 1 to 5 doses. In contrast, n-hexane was “judged negative” in this test by Linder et al. (1992).

n-Hexane is chemically distinct from its listed metabolites, and the fact that it is metabolized to many substances, including two Proposition 65 listed chemicals, cannot alone be the deciding factor to list it. Indeed, as discussed elsewhere in these comments, exposure to n-hexane does not result in levels of exposures to these metabolites at levels that cause such effects.

V. Human Studies Do Not Support a Finding that n-Hexane Is Clearly Shown to Cause Reproductive Toxicity

As noted in the draft HID, “[n]o studies in OEHHA’s literature search were identified regarding reproductive effects in humans after exposure to n-hexane.”¹⁰

VI. Animal Studies Do Not Support a Finding that n-Hexane Is Clearly Shown to Cause Reproductive Toxicity

The draft HID identifies a large number of studies in animals of n-hexane relevant to developmental and reproductive toxicity. The quality and reliability of these studies vary, rendering most of them incompatible with the statutory standard for listing, i.e., they do not constitute the statutorily required “scientifically valid testing in accordance with generally accepted principles.” Such studies cannot establish that n-hexane is “clearly shown” to cause reproductive toxicity.

This section includes a brief evaluation of each of the animal studies identified in Tables 1-3 of the draft HID. The draft HID identifies ten studies relevant to developmental toxicity in Table 1, four studies relevant to female reproductive toxicity in Table 2, and seven studies relevant to male reproductive toxicity in Table 3. There are actually only seventeen studies in total identified in Tables 1-3 because four studies appear in more than one table. For example, two developmental toxicity studies by Li et al. (2014, 2015) appear in both Tables 1 (developmental toxicity) and 2 (female reproductive toxicity). Similarly, two male dominant lethal studies (Litton Bionetics, 1980; Mast et al., 1988c) may be found in both Tables 1 (developmental toxicity) and 3 (male reproductive toxicity).

¹⁰ OEHHA (2017) Draft Hazard Identification Document, Consideration of n-Hexane for Listing under Proposition 65 as Known to Cause Reproductive Toxicity, September, 2017, p. 5.

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Some of the studies identified in the draft HID do not constitute “scientifically valid testing according to generally accepted principles.” Examples of these critical deficiencies include: unknown composition of the test material, inadequate group size, insufficient number of doses to evaluate a dose-response relationship, lack of detail with regard to both methods and results, and improper statistical analysis (not on a per litter basis). Because they fail to meet the statutory standard, these studies should be afforded no weight in determining whether n-hexane meets the listing criteria.

The results of the animal studies do not support a conclusion that n-hexane is “clearly shown” to cause developmental toxicity, male reproductive toxicity, or female reproductive toxicity. Each of these endpoints is discussed separately in the sections below.

1. Developmental Toxicity

Table 1 of the draft HID identifies 10 studies on the developmental toxicity of n-hexane, including six inhalation developmental toxicity studies in rats, two developmental toxicity studies in mice (one inhalation and one oral), and two inhalation dominant lethal studies in male mice. The developmental toxicity studies in animals do not “clearly show, through scientifically valid testing according to generally accepted principles” that n-hexane causes developmental toxicity.

In the developmental toxicity studies in rats and mice, n-hexane showed little or no evidence of developmental toxicity except at doses that produced overt maternal toxicity and even death. Two dominant lethal assays of inhaled n-hexane in male mice revealed no evidence of a dominant lethal effect. Each of the ten studies identified in Table 1 of the draft HID are addressed in the sections below.

Developmental Toxicity Studies in Rats

Bus et al. (1979) “In a study on the effect of n-hexane exposure at various times during gestation, no significant adverse developmental effects were found.”¹¹ Groups of pregnant F344 rats (n = 7 group) were exposed to 0 or 1000 ppm of n-hexane, 6 hours/day, on GD 8-12, 12-16, or 8-16 (Bus et al., 1979). No significant alterations in fetal resorptions, body weights, external, soft tissue or skeletal anomalies were observed in any of the treatment groups.

A low incidence (not statistically significant) of pyelectasis (enlarged renal pelvis) was observed in each of the three treatment groups, which was observed only when litters contained fewer than three fetuses. However, no increase in pyelectasis was seen in subsequent developmental toxicity studies by NIEHS and others using larger numbers of rats and higher exposures to n-hexane, as described below.

Some pregnant females exposed on GD 8-16 were permitted to deliver their offspring, and total litter body weights and mortality were monitored at weekly intervals up to 7 weeks after parturition. A temporary decrease in pup weight gain (14% decrease for up to 3 weeks after birth) was seen in the offspring of dams exposed on GD 8-16; however, body weights reached levels similar to controls after 7 weeks. The study authors concluded that n-hexane had little effect on reproduction and development of F344 rats.

Litton Bionetics (1979) In another developmental toxicity, pregnant Sprague-Dawley rats were exposed by inhalation to 0, 93 or 409 ppm n-hexane for 6 hours/day on GD 6-15. This study employed adequate numbers of pregnant rats (n =20/group). US EPA evaluated this study and concluded, “no n-hexane-related effects were observed.”¹² No evidence of maternal toxicity was reported. N-hexane did not significantly affect resorptions, mean litter size, and mean fetal body weight. No statistically significant

¹¹ ATSDR (1999) Toxicological Profile for n-Hexane. p. 55.

¹² US EPA (2005) Toxicological Review of n-Hexane. EPA/635/R-03/012. p. 59.

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difference in the incidences of external, soft tissue and skeletal abnormalities was observed between control and treatment groups.

Mast (1987) In a conventional developmental toxicity study conducted by NIEHS at Battelle Pacific Northwest Laboratory, groups of pregnant Sprague-Dawley rats (n = 30/group) were exposed by inhalation for 20 hours/day to 0, 200, 1000, or 5000 ppm n-hexane on GD 6-19 (Mast, 1987). Due to the long daily duration of exposure (20 hours/day), feed was left in place with the rats for 24 hours/day and replaced daily. With this experimental design, the animals may have been exposed orally to some amount of n-hexane through contamination of the feed inside the inhalation chamber. Significantly, the contamination of the feed may have also created an aversion to the taste of the feed, which may explain why such drastic reductions in maternal body weight gain were observed in this study compared to the studies where inhalation exposure was for shorter daily durations (and the feed was not placed in the inhalation chamber).

Significant treatment-related maternal toxicity was observed in the form of decreased maternal body weight and body weight gain. Maternal body weight was statistically significantly decreased among dams at 5000 ppm as measured on GD 8 and GD 20 (the day of necropsy). Extra-gestational maternal weight gain (defined as the body weight minus the gravid uterine weight at sacrifice on GD20 compared to the body weight on GD 0) was reduced by 20%, 23%, and 45% at 200, 1000, and 5000 ppm, respectively; the difference was statistically significant at 5000 ppm.

There was no effect of n-hexane on implantations, resorptions, live fetuses, fetal sex ratio and malformations at any exposure level. Fetal body weight was statistically significantly decreased among male fetuses at 1000 and 5000 ppm (7% and 15%, respectively) compared to controls. In females, fetal body weight was reduced compared to controls at 1000 and 5000 ppm (3% and 14%, respectively); the decrease was statistically significant at 5000 ppm only. Also, at 5000 ppm, there was a statistically significant increase in the incidence of reduced ossification of sternbrae 1-4 per litter compared to controls (14% and 39% at 0 and 5000 ppm, respectively). An increased

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incidence of reduced ossification of sternbrae is expected when fetal body weights are 14-15% less than those of the control group. Importantly, both the decreased fetal body weight and the decreased ossification of sternbrae 1-4 at 5000 ppm are easily explained by a 45% decrease in extra-gestational maternal weight gain (i.e., minus the gravid uterine weight) at 5000 ppm. Based on the data in Table 5 of Mast (1987), the decrease in fetal body weight (as measured by the gravid uterine weight) accounted for only 30% (9.7/32.2 grams) of the decrease in maternal weight gain observed at 5000 ppm. Similarly, the 7% decrease in male fetal body weight at 1000 ppm is also consistent with the observed maternal toxicity (i.e., 23% decrease in extra-gestational maternal body weight gain). In summary, Mast (1987) observed decreased fetal body weight at a dose that caused excessive maternal toxicity. The excessive maternal toxicity observed in this study renders it unacceptable for evaluation of n-hexane as a developmental toxicant under the statutory listing requirement of “scientifically valid testing according to generally accepted principles.”

Stoltenburg-Didinger et al. (1990, 1991) The developmental toxicity of n-hexane was evaluated in female Wistar rats exposed by inhalation for 23 hours/day for 21 consecutive days. The 21-day exposure period is assumed to be the 21 days during pregnancy, but the exposure period is not clearly defined by the study authors, a critical deficiency that renders the study unacceptable under the statutory listing standard of “scientifically valid testing according to generally accepted principles” and under the DARTIC’s own Guidance Criteria with respect to experimental design. Three experiments were performed using a control group and only one concentration of n-hexane: either 500, 800 or 1500/1000 ppm. Since each dose level was run at a different time, it is not possible to evaluate dose-response, another critical study deficiency. Further, in the case of the 1000 ppm group, the pregnant rats were initially exposed to 1500 ppm, but at some time point the concentration was reduced to 1000 ppm. The study authors do not describe which day or at which time point the exposure concentration was changed – another critical deficiency.

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Prenatal exposure to n-hexane did not cause neurotoxicity among the dams or their offspring at any exposure concentration. The authors reported “the pregnancy rate and the derived resorption rate showed a marked relation to the exposure concentration.” However, this statement is confusing and misleading. The pregnancy rate was slightly higher at 500 ppm compared to its concurrent control group (10/16 and 15/20 at 0 and 500 ppm, respectively). At 800 and 1500/1000 ppm, the group size (n= 8) was too small to detect a difference in the pregnancy rate between their concurrent control groups. No statistical evaluation was performed. Inexplicably, the study authors reported a *decrease* in the resorption rate at increasing concentrations of exposure to n-hexane.

Stoltenburg-Didinger et al. reported a decrease in birth weight among the offspring of pregnant rats exposed to 500 ppm, but not at 800 or 1500/1000 ppm. Again, no statistical evaluation of birth weight was performed, a deficiency in experimental design and analysis. There did not appear to be any significant difference in brain weight relative to body weight among the offspring of rats exposed to any concentration of n-hexane.

A delay in the histiogenesis of the cerebellar cortex was reported for all solvents studied, including n-hexane, among the offspring of dams exposed to all concentrations. However, in the case of the dams exposed prenatally only (not prenatally and postnatally), this delay was reversible: “The animals exposed prenatally no longer showed any appreciable differences on day 30 pn; only the experimental animals submitted to further continuous exposure showed a thinner molecular layer and persistence of an outer granular layer on day 30 after birth.”¹³

In summary, this study was poorly reported and suffered from many critical deficiencies rendering it unacceptable for evaluation because it fails to meet the statutory listing standard of “scientifically valid testing according to generally accepted principles,” and fails to meet the Guidance Criteria for experimental design and dose levels. Even if the DARTIC were to accept this study as meeting the statutory and Guidance Criteria, the

¹³ Stoltenburg-Didinger et al. (1990) p. 587.

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study provides little evidence of developmental toxicity. For all these reasons, n-hexane is not “clearly shown” to cause developmental toxicity.

Li et al. (2014) The developmental toxicity of n-hexane was investigated in pregnant rats exposed for 4 hours/day on GD 1-20 to 0, 500, 2500 or 12,500 ppm. A major deficiency of this study, rendering it unsuitable for evaluation under the statutory listing standard and the Guidance Criteria, is the small group size of 5 rats per group. Maternal toxicity was observed at 12,500 ppm; the study stated the 12,500 ppm exposed dams “showed obvious mental symptoms, such as irritability and an attack tendency.” No other symptoms of maternal toxicity were reported, but it is not clear whether standard endpoints of maternal toxicity (e.g., body weight, food consumption) were evaluated beyond clinical symptoms. This study is unacceptable for evaluation because the absence of standard endpoints renders it not “scientifically valid testing according to generally accepted principles.”

No malformations were found in any of the living pups, and no significant difference in pup weight was observed among the exposed groups compared to the controls. Among the offspring of dams exposed to 12,500 ppm, there was a decrease in live pups per litter. A decrease in proportion of secondary follicles and an increase in the proportion of atresic follicles were reported at 12,500 ppm, but not at the lower concentrations.

In summary, this study was poorly reported and suffers from too many deficiencies (e.g., group size of 5 rats/group) to be considered “scientifically valid testing according to generally accepted principles.” Even if the study were deemed acceptable, it found limited evidence of developmental toxicity at a dose that caused “obvious mental symptoms” among the dams. For all these reasons, the study fails to demonstrate that n-hexane is “clearly shown” to cause developmental toxicity.

Li et al. (2015) The same investigators conducted a second developmental toxicity study in Wistar rats that is similar in experimental design to Li et al. (2014). Small groups of pregnant rats (n = 6 rats/group) were exposed by inhalation to 0, 100, 500, 2500, or

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12,500 ppm n-hexane for 4 hours/day on GD 1-20. Maternal toxicity was not assessed. There were no significant differences in pup body weight, vaginal opening, and ovarian histopathology between control and exposed pups. As in the previous study, the mean number of live pups per litter was statistically significantly reduced at 12,500 ppm. Compared to the control group, the female offspring of dams exposed to 12,500 ppm n-hexane had significantly shortened diestrus; groups exposed to 500 and 2500 ppm n-hexane exhibited significantly prolonged estrus; groups exposed to 100 and 500 ppm had significantly prolonged proestrus. The estrus cycle was monitored over a period of two estrus cycles; because of the normal variation in the stages of the estrus cycle in rats, the estrus cycle is usually monitored over three estrus cycles. The authors opined that the differences in results at different dose levels was due to suppressed secretion of progesterone at low doses and expression of hormone synthesis-related genes at the high dose.

This study reports some evidence of developmental toxicity in the offspring of dams exposed to n-hexane. However, due to critical deficiencies, the study fails to meet the statutory listing requirement of “scientifically valid testing according to generally accepted principles.” Deficiencies of this study include the small group size (n = 6 rats per group) and no measurements of maternal toxicity. The absence of any maternal toxicity assessment is particularly notable, because in Li’s 2014 study, the same high dose exposure to 12,500 ppm was reported to cause “obvious mental symptoms.” Based on those prior results, it is likely that maternal toxicity was a significant factor in the observations made in this study.

Developmental Toxicity Studies in Mice

Marks (1980) Marks et al. (1980) conducted a developmental/reproductive toxicity study in CD-1 mice administered n-hexane in cottonseed oil by gavage on GD 6-15. In the first portion of this study, groups of 6-37 mice were given 0 (37 mice), 260 (13 mice), 660 (6 mice), 1320 (6 mice), or 2200 (14 mice) mg/kg/day of n-hexane. There were no

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developmental or reproductive effects with n-hexane observed in this portion of the study.

In the second portion of the study, groups of 24-33 pregnant mice received cumulative doses of 0 (24 mice), 1830 (24 mice), 2170 (25 mice), 7920 (34 mice), or 9900 (33 mice) mg/kg/day on GD 6-15 in the form of three separate gavage doses spaced throughout the day. Maternal mortality was observed at all but the lowest dose group. The incidence of maternal deaths was 0/24, 0/24, 2/25, 3/34, and 5/33 at 0, 1830, 2170, 7920 and 9900 mg/kg/day, respectively. Fetal body weight was statistically significantly decreased (6% decrease) among the offspring of mice given 7920 or 9900 mg/kg/day. There was no effect on the incidence of resorptions or the mean number of live pups per litter. No significant increase in fetal malformations or variations was observed at any dose level, including dose levels that caused overt maternal toxicity and death.

In summary, this study showed little evidence of developmental toxicity at dose levels that caused overt maternal toxicity, including death.

Mast et al. (1988a) Mast et al. (1988a) conducted an inhalation developmental toxicity study of n-hexane in CD-1 mice. Groups of pregnant CD-1 mice (n = 30 mice/group) were exposed to 0, 200, 1000 or 5000 ppm n-hexane for 20 hours/day on GD 6-17. Statistically significant decreases in maternal weight gain and relative uterus weight were observed among dams exposed to 5000 ppm. At 5000 ppm, the number of live fetuses per litter and fetal body weight (females only) were statistically significantly reduced, and the number of late resorptions was statistically significantly increased. There was also an increase in the incidence of exencephaly at 5000 ppm; however, the study authors at NTP did not consider this to be a treatment-related effect due to the lab's background incidence of this anomaly. According to ATSDR, "No effects were seen at 200 or 1000 ppm in this study."¹⁴

¹⁴ ATSDR (1999) p. 52.

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In summary, inhalation exposure to n-hexane for 20 hours/day produced developmental toxicity at a concentration (5000 ppm) that produced maternal toxicity, but not at concentrations that did not produce maternal toxicity.

Dominant Lethal Studies in Mice

The draft HID identified two dominant lethal studies in male CD-1 mice exposed to n-hexane by inhalation. A dominant lethal effect was not seen in either study.

Litton Bionetics (1980) In a dominant lethal assay, male CD-1 mice were exposed to 0, 99, or 396 ppm n-hexane for 6 hours/day for 5 days/week for 8 weeks. No evidence of a dominant lethal effect was found. The average number of implantations per pregnant female (non-exposed) was not affected by n-hexane exposure to the males. The average number of dead or resorbed implantations was not statistically significantly increased by paternal exposure to n-hexane. Comparison between the proportions of females with one or more dead implant also showed no adverse effect from n-hexane exposure.

Mast et al. (1988b) Mast et al. (1988b) conducted a dominant lethal study of inhaled n-hexane in male CD-1 mice. Groups of 20 male mice were exposed to 0, 200, 1000 or 5000 ppm n-hexane for 20 hours/day for 5 consecutive days. Mated females were sacrificed 12 days after the last day of cohabitation, and their reproductive status and their numbers and viability of their implants were recorded. According to US EPA, “The number of live implants was consistently greater than 10 fetuses/litter, and there was no indication of a decline in the reproductive index as a result of increasing n-hexane exposure in the males. Furthermore, there was no increase in the number of dead implants or early resorptions as a result of the males being exposed to n-hexane prior to mating. The study authors concluded that short-term exposure to n-hexane vapor did not result in a male dominant lethal effect in CD-1 mice.”¹⁵

¹⁵ US EPA (2005) p. 62.

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In summary, neither the developmental toxicity studies nor the dominant lethal studies, considered individually or collectively, “clearly show through scientifically valid testing according to generally accepted principles” that n-hexane causes developmental toxicity.

2. Female Reproductive Toxicity

Table 2 of the draft HID identified four studies of female reproductive toxicity involving exposure to n-hexane (Liu et al. 2012, 2013; Li et al., 2014, 2015). All four of these studies were conducted by the same group of investigators at the Fujian Health College, Fujian, China.

It is difficult to compare the results of these studies for consistency of results because the experimental design and endpoints evaluated differed from study to study. For example, while most were inhalation studies, the duration of exposure ranged from 4 to 8 hours/day and from 7 days to 5 weeks. Two studies were developmental toxicity studies in pregnant rats; both of these studies appear in both Table 1 (developmental toxicity studies) and Table 2 (female reproductive toxicity studies) of the draft HID. The other two studies were studies in non-pregnant mice. The exposure concentrations were different in every study. And finally, in most cases, the endpoints evaluated were different, limiting the ability to evaluate consistency of results across studies.

Systemic toxicity was assessed in only two studies, and when systemic toxicity was assessed, it was significant. For example, Liu et al. (2012) observed that mice in each treatment group “appeared to be quiet to different degrees.” At the highest exposure concentration (approximately 20,000 ppm n-hexane), systemic toxicity included decreased body weight, decreased activity, decreased depilation, decreased appetite, rhabdomyolysis, “ulcers in the abdominal area,” and one death. In comparison, Li et al. (2014) reported that pregnant rats exposed to 12,500 ppm n-hexane “had mental symptoms: irritability and an attack tendency.” Apparently, Li et al. (2014) did not measure body weight or food consumption. The studies by Liu et al. (2013) and Li et al. (2015) did not assess systemic toxicity at all; yet, Liu et al. (2013) used a higher

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concentration of n-hexane (approximately 24,000 ppm) than any of the studies which reported significant systemic toxicity.

It is a generally accepted principle that studies like these should assess systemic toxicity, as well as reproductive toxicity. Indeed, consideration of systemic toxicity is a specific criterion under the DARTIC Guidance Criteria. This study deficiency, which makes it difficult to determine what role systemic toxicity played in the effects reported among female rats, means that the study fails to meet the statutory listing standard of “scientifically valid testing according to generally accepted principles.”

The two developmental toxicity studies identified in Table 2 of the draft HID used inadequate numbers of pregnant rats to assess endpoints of developmental toxicity, including endpoints of female reproductive toxicity among the offspring. The group size was only 5 rats/group and 6 rats/group in the Li et al. (2014) and Li et al. (2015) studies, respectively. Furthermore, the investigators did not use an appropriate statistical analysis for much of the data. For example, in the Li et al. (2015) study, two female pups from each of six litters were evaluated for most endpoints, and it appears the statistical unit chosen was 12 female pups/group. The correct statistical unit should have been the litter, and there were only 6 litters/group. The statistical analysis should have been performed on the mean of the 6 litter means. Therefore, the studies fail to meet the statutory listing criterion of “scientifically valid testing according to generally accepted principles.”

Even in the few cases where similar endpoints were evaluated in two different studies, consistency of results cannot be assessed fully. For example, in the Liu et al. (2012) study, serum progesterone levels were reported to be significantly reduced at all exposure concentrations (approximately 800, 4000 and 20,000 ppm). In comparison, Li et al. (2015) measured progesterone in the supernatants of granulosa cells, and found that progesterone was significantly decreased at the highest concentration (12,500 ppm) and increased at concentrations of 100, 500 and 2500 ppm.

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Finally, the DARTIC evaluated both 2,5-HD and MnBK for evidence of female reproductive toxicity, and neither were listed as female reproductive toxicants. The evidence that n-hexane causes female reproductive toxicity is no more compelling than the evidence that MnBK or 2,5-HD cause female reproductive toxicity.

3. Male Reproductive Toxicity

The studies addressing male reproductive toxicity do not provide evidence that n-hexane is “clearly shown ... to cause” that harm. It is well known that n-hexane is metabolized to MnBK and 2,5-HD, as well as other metabolites. Both of these chemicals appear on the Proposition 65 list as male reproductive toxicants. However, it is overly simplistic to assume that n-hexane is “clearly shown” to cause male reproductive toxicity simply on this basis. The potential for n-hexane to produce reproductive toxicity is likely to depend on whether the internal dose (peak levels and area under the curve at the target site) of these metabolites is sufficient to cause male reproductive toxicity.

There is a clear difference between n-hexane and 2,5-HD regarding the potential to cause male reproductive toxicity. Scientists at US EPA conducted a short-term screening assay for spermatotoxicity, and both n-hexane and 2,5-HD were evaluated in the same assay under the same conditions (Linder et al., 1992). The results were markedly different. For this test, n-hexane or 2,5-HD were given to male rats orally by gavage twice daily for a total daily dose of 20,000 mg/kg/day. Exposure to 2,5-HD produced substantial spermatotoxicity after 1 to 5 doses. In contrast, n-hexane was “judged negative” in this test by Linder et al. (1992).

Other studies of n-hexane demonstrate inconsistent evidence of male reproductive toxicity, which was observed only at dose levels that produce serious toxicity.

DeMartino et al. (1987) showed adverse effects on spermatogenesis among rats exposed to 5000 ppm n-hexane for 16 hours/day for up to six weeks, i.e., exposures associated with significant signs of toxicity, including neuropathy and severe weight loss serious

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enough to warrant premature sacrifice of some animals. In contrast, no increased incidence of spermatotoxicity was observed in the absence of systemic toxicity among mice exposed to up to 5000 ppm n-hexane for 20 hours/day for five consecutive days (Mast et al., 1988c). Nylen et al. (1989) found atrophic changes in the seminiferous tubules among rats exposed to 1000 ppm n-hexane for 18-21 hours/day for 28 or 61 days; however, all rats that exhibited testicular damage also demonstrated severe atrophy of the muscles of the hindlimbs and reduced body weight. Rats (n = 6 rats/group) exposed to 1000 ppm n-hexane for 4 hours/day for 6 days/week for 415 days found an increased incidence of Leydig cell hyperplasia; however, no changes in spermatogenesis were reported and fertility was not evaluated.

Table 3 of the draft HID identifies 7 studies providing data on the potential male reproductive toxicity of n-hexane. Each of these studies is described below in the order in which they appear in Table 3 of the draft HID.

DeMartino et al., (1987) DeMartino et al. (1987) conducted a male reproductive toxicity study of n-hexane in Sprague-Dawley rats exposed by inhalation. Groups of male rats were exposed to a single exposure concentration of 5000 ppm n-hexane in either: (1) a single 24-hour exposure, (2) repeated 16-hour/day exposures for up to 8 days, or (3) repeated 16-hour/day exposures for 6 days per week for up to 6 weeks. Treated rats were allowed to recover from exposure to n-hexane for varying lengths of time, ranging from 2 days to 29 weeks, after the last day of exposure. Rats exposed to 5000 ppm n-hexane displayed some evidence of neuropathy such as paralysis, and extreme cases were sacrificed moribund and necropsied rather than being allowed to die and undergo autolysis.

The earliest lesions occurred after a single 24-hour exposure, involving focal degeneration of primary spermatocytes and cytoplasmic swelling of spermatids. The rats recovered completely from these lesions in 2-4 weeks after a single 24-hour exposure to 5000 ppm n-hexane.

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In comparison, rats exposed to 5000 ppm for 16 hours/day over a 6-week period exhibited complete atrophy of the seminiferous tubules. There was also a wide range of testicular effects that did not completely reverse during the recovery period. Most rats exposed for up to 6 weeks showed signs of polyneuropathy, as well as decreased body weight between the first and sixth weeks of exposure and food consumption (30% decrease).

In summary, this study showed adverse effects on spermatogenesis, which increased with longer durations of exposure. However, the study examined only one dose level, and the single concentration tested, 5000 ppm, was associated with significant signs of toxicity, including neuropathy and severe weight loss, which was serious enough to warrant premature sacrifice of some animals.

Litton Bionetics (1980) This negative dominant lethal assay was described earlier in the section on developmental toxicity. Male CD-1 mice were exposed to 0, 99 or 396 ppm n-hexane for 6 hours/day, 5 days/week for 8 weeks. The study meets the Guidance Criteria and should be considered “scientifically valid testing according to generally accepted principles.” According to both the draft HID and the ATSDR (1999) evaluation, there was no adverse effect on male fertility or any other endpoint evaluated in this study.

Mast et al. (1988b) This second negative dominant lethal assay was discussed previously in the Developmental Toxicity section. As noted earlier, there was no evidence of any male reproductive toxicity in male CD-1 mice exposed to up to 5000 ppm n-hexane for 20 hours/day for five consecutive days. According to US EPA, “The number of live implants was consistently greater than 10 fetuses/litter, and there was no indication of a decline in reproductive index as a result of increasing n-hexane exposure in the males. Furthermore, there was no increase in the number of dead implants or early resorptions as a result of the males being exposed to n-hexane prior to mating. The study authors concluded that short-term exposure to n-hexane vapor did not result in a male dominant lethal effect in CD-1 mice.”¹⁶

¹⁶ US EPA (2005) p. 62.

Mast et al. (1988c) This is another negative study for male reproductive toxicity. According to US EPA (2005), “A detailed study by Mast et al. [1988c] examined the effects of n-hexane on sperm morphology in B6C3F1 mice. The experimental program featured the exposure of 20 male B6C3F1 mice/group to 0, 200, 1000, or 5000 ppm n-hexane, 20 hours/day for 5 consecutive days, after which the animals were examined for gross lesions of the reproductive tract and any disturbances in sperm morphology. The study meets the Guidance Criteria and should be considered “scientifically valid testing according to generally accepted principles.” There were no clinical signs of toxicity or body weight changes in any of the dose groups, nor was there an increased incidence of aberrant sperm characteristics such as blunt-hook, banana, amorphous, or pin-head shapes. Likewise, there was no increased incidence of sperm with more than one head or tail.”¹⁷

Nylen et al. (1989) Male reproductive effects with loss of nerve growth factor were observed in rats exposed to 1000 ppm n-hexane by inhalation for up to two months; these exposures also caused marked systemic toxicity. In this study by Nylen et al. (1989), Sprague-Dawley rats were exposed to 0 or 1000 ppm n-hexane for 21 hours/day for 28 days or for 18 hours/day for 61 days. Atrophic changes in the seminiferous tubules throughout the testes were evident at 2 weeks, as well as 10, 12, and 14 months after exposure. Importantly, all of the rats that exhibited testicular damage also demonstrated severe atrophy of the muscles of the hindlimbs (presumably due to hindlimb paralysis). Other signs of toxicity included reduced body weight; however, the extent of the body weight loss was not reported.

Linder et al. (1992) Investigators at US EPA evaluated 14 chemicals, including n-hexane and 2,5-HD, in a short-term screening assay for spermatotoxicity using 4-6 rats/test compound. For this test, n-hexane, 2,5-HD, or 12 other chemicals were given orally by gavage twice daily for a total daily dose of 20,000 mg/kg/day. 2,5-HD and five

¹⁷ US EPA (2005) p. 62.

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other chemicals produced substantial spermatotoxicity after 1 to 5 doses. In contrast, n-hexane was “judged negative” in this test by the US EPA scientists.

Imai and Omoto (1999) This study is published as a 2-page “Short Communication.” Imao and Omoto exposed groups of male F344 rats (n = 6 rats/group) to 0 or 1000 ppm of n-hexane for 4 hours/day for 6 days/week for 415 days. According to the study authors, “Body weight, testes weight, food intake, the frequency of 14 cellular associations in the seminiferous tubules [according to the standard criteria of Leblond and Perey] and light microscopical histologic findings in a single testis of the exposed group did not significantly differ from the controls, but the incidences of Leydig cell hyperplasia and Leydig cell tumor (Figure IA) in the 6 rats exposed to n-hexane were 100% (6/6) and 33% (2/6), respectively. In contrast, the incidences of Leydig cell hyperplasia (Fig. 1B) and Leydig cell tumor in the control group of 6 rats were only 16.7% (1/6) and 0%.”¹⁸ Of note, F344 rats are extremely susceptible to Leydig cell hyperplasia and tumors. Close to 100% of male F344 rats exhibit Leydig cell tumors at the end of a 2-year study.

The study authors also state: “there are no morphologic differences between Leydig cell hyperplasia and Leydig cell tumors, according to the National Toxicology Program.” This statement suggests that the authors may not have accurately distinguished between Leydig cell hyperplasia and tumors. NTP does provide morphologic criteria:

Leydig cell adenoma begins as hyperplasia and consists of one or more foci of expansile LCs that typically compress adjacent tubules. The distinction between hyperplasia and adenoma is difficult as cellular characteristics cannot distinguish between them. Most often size is used as a morphologic criteria to differentiate hyperplasia from adenoma. One recommendation from the National Toxicologic Program (NTP) is to use the size of adjacent tubules as a threshold. When the lesion grows larger than the diameter of the adjacent tubule, it is diagnosed as adenoma. In 2005 toxicologic pathology societies from around the world began an

¹⁸ Imai and Omoto (1999) p. 261.

initiative to standardize nomenclature for proliferative and nonproliferative lesions in rats and mice. This initiative is termed the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (INHAND) (Mann et al., 2012). The INHAND focuses on specific organ systems. For the male reproductive system, the INHAND guidance differentiates hyperplasia from adenoma using a size of three seminiferous tubules, among other criteria (Creasy et al., 2012). This makes interpretation of incidence data in rodents difficult as published data on LC adenoma typically does not describe the methodology used in establishing the diagnosis of adenoma (Cook et al., 1999).¹⁹

In summary, this study suggests that chronic exposure to n-hexane in F344 rats may damage the testes by increasing the incidence of Leydig cell hyperplasia. However, it appears that the authors may not have accurately distinguished between Leydig cell hyperplasia and tumors, and no changes in spermatogenesis were reported. In addition, it is well known that this rat strain is susceptible to Leydig cell hyperplasia and tumors, which confuses the study results. Fertility of these rats was not evaluated.

In summary, the male reproductive toxicity studies, considered individually or collectively, do not “clearly show through scientifically valid testing according to generally accepted principles” that n-hexane causes male reproductive toxicity.

VII. Conclusion

There are no relevant epidemiological studies of the reproductive toxicity of n-hexane, and none were identified in the draft HID. In comparison, the draft HID identifies 10, 4, and 7 animal studies that have evaluated the potential developmental toxicity, female reproductive toxicity and male reproductive toxicity of n-hexane. The quality and reliability of these studies vary, as noted in the Experimental Parameters section of

¹⁹ Steinbach TJ, Maronpot RR , Hardisty, JF (2017) Human relevance of rodent Leydig cell tumors. <https://focusontoxpath.com/articles/HUMAN-RELEVANCE-OF-RODENT-LEYDIG-CELL-TUMORS.pdf>

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Tables 1-3 in the draft HID. While two metabolites of n-hexane (i.e., MnBK and 2,5-HD) have been identified as causing male reproductive toxicity, that fact alone cannot meet the stringent standard for listing the parent substance n-hexane. In fact, a short-term screening study by US EPA in rats given 20,000 mg/kg/day of each test material for 5 days showed clear evidence of male reproductive toxicity with 2,5-HD, but not with n-hexane. The evidence of male reproductive toxicity is unclear with n-hexane, and in those studies where male reproductive effects are reported, such effects are seen in the presence of significant systemic toxicity. For the reasons detailed in these comments, the overall scientific evidence does not support a conclusion that n-hexane has been “clearly shown” to cause developmental toxicity, female reproductive toxicity or male reproductive toxicity.

VIII. References

The references cited in this submission are fully identified in the References section of the draft HID.