OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

Consideration of *n*-Hexane for Listing under Proposition 65 as Known to Cause Reproductive Toxicity

September 2017

Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Contents

Introduction

Proposition 65[1] requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency maintains this list in its role as the lead agency for implementation of Proposition 65[2]. This document presents information on the reproductive toxicity of n-hexane, for consideration by the Developmental and Reproductive Toxicant Identification Committee (DARTIC), the state's qualified experts for reproductive toxicity under Proposition 65. At a meeting scheduled for November 29, 2017, the DARTIC will be considering whether *n*-hexane has been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity and should be placed on the Proposition 65 list.

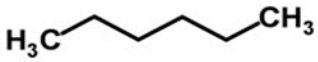
History

At a meeting held on November 9, 2015[3] the DARTIC reaffirmed the listing of methyl-n-butyl ketone (MnBK) as a chemical known to the state to cause reproductive toxicity on the basis of male reproductive toxicity and determined that an additional endpoint, developmental toxicity, should be identified. At that meeting, the DARTIC also determined that 2,5-hexanedione (2,5-HD), a metabolite of MnBK, had been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity, based on the male reproductive endpoint.

At the November 9, 2015 meeting, the DARTIC requested that OEHHA bring *n*-hexane before the committee at a future meeting. The request was made because *n*-hexane is metabolized to MnBK and 2,5-HD and thus it was considered to be inextricably linked toxicologically with MnBK and 2,5-HD.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986, codified at Health and Safety Code section 25249.5 *et seq.,* commonly referred to as Proposition 65.
[2] Health and Safety Code section 25249.12, Title 27, Cal. Code of Regs., section 25102(o)
[3] Meeting transcript available at https://oehha.ca.gov/media/downloads/proposition-65/transcript/11092015oehhadartictranscript.pdf

Chemical Identity



n-Hexane

IUPAC name: Hexane
Molecular formula: $CH_3(CH_2)_4CH_3$

n-Hexane is a widely used industrial solvent present in varnishes, cements, glues, and inks. It has also been used as an agent to extract natural oils from various seeds, including cotton and soybean seeds.

Literature Search and n-Hexane Data Tabulation

OEHHA, through a contract with the Sheldon Margen Public Health Library at the University of California, Berkeley, conducted literature searches to identify studies that potentially provide information on the reproductive toxicity of *n*-hexane. The search strategy applied is provided in Appendix A. The searches covered the three major reproductive toxicity endpoints; namely, developmental toxicity, male reproductive toxicity and female reproductive toxicity. Additionally, a search was conducted to identify studies that describe the metabolism of *n*-hexane.

OEHHA staff reviewed the results of these searches and identified all studies that provided data on reproductive toxicity of *n*-hexane following direct exposure to the compound. The design parameters and results of these studies on male reproductive, female reproductive and developmental toxicity are summarized in this document in separate tables for each endpoint, and the study reports have been provided to the DARTIC and are available to the public upon request.

Reproductive Toxicity Data on n-Hexane Metabolites

Information on the metabolism of *n*-hexane is also provided in this document. Because *n*-hexane is metabolized to MnBK and 2,5-HD in the body, data on the reproductive and developmental toxicity of MnBK and 2,5-HD is relevant to the potential identification of *n*-hexane as causing reproductive toxicity under Proposition 65. The DARTIC is therefore also being provided with the 2015 OEHHA hazard identification document "Reconsideration of Methyl n-Butyl Ketone Listed under Proposition 65 as Known to Cause Reproductive Toxicity (Chemical Listed via the Labor Code Mechanism) 2015 Update and Consideration of 2,5-Hexanedione for Listing under Proposition 65 as

Known to Cause Reproductive Toxicity". That document is provided here as Attachment 1.

Commercial Hexane

Information on commercial hexane was brought to OEHHA's attention as a result of the Request for Relevant Information on *n*-hexane[4]. Commercial hexane is a complex mixture comprised of six carbon isomers, and consists of *n*-hexane (approximately 40-50%) and about 12-16% each of 3-methylpentane, methylcyclopentane and 2-methylpentane (NTP, 1991; Daughtrey *et al.*, 1994). Since the DARTIC will be considering whether *n*-hexane has been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity, only studies of *n*-hexane itself are presented here. Studies of complex mixtures containing *n*-hexane, such as commercial hexane, are not presented here.

n-Hexane Metabolism to Methyl n-Butyl Ketone and 2,5-Hexanedione

As noted by the US Environmental Protection Agency (US EPA): "*n*-hexane is a precursor to 2-hexanone [MnBK] and both compounds can be further metabolized to form the highly toxic compound 2,5-HD" (USEPA, 2009).

The metabolism of *n*-hexane to form MnBK (also known as 2-hexanone) and 2,5-HD *n*-hexane has been summarized by the US EPA (USEPA, 2009), the National Toxicology Program, (NTP, 1991) and the Agency for Toxic Substances and Disease Registry (ATSDR, 1999). Multiple reviews have also summarized the findings from metabolism studies that demonstrate that 2,5-HD is the predominant metabolite of both MnBK and *n*-hexane (Krasavage *et al.*, 1980; Couri and Milks, 1982; Boekelheide and Schoenfeld, 2001; Boekelheide *et al.*, 2003). The metabolic relationship between MnBK and 2,5-HD is also described in the 2015 OEHHA hazard identification document for those chemicals (Attachment 1).

Briefly, studies in guinea pigs and rats have demonstrated that MnBK and *n*-hexane share common metabolic pathways and give rise to common metabolites (Abdel-Rahman *et al.*, 1976; DiVincenzo *et al.*, 1976). The predominant metabolite identified in serum is 2,5-HD (DiVincenzo *et al.*, 1976). Urinary metabolites of *n*-hexane and MnBK include 2-hexanol, 2,5-hexanediol, 5-hydroxy-2-hexanone (Abdel-Rahman *et al.*, 1976; Couri *et al.*, 1978; Eben *et al.*, 1979; Hamelin *et al.*, 2005) . In inhalation studies in

[4] Available at https://oehha.ca.gov/proposition-65/crnr/chemicals-selected-oehha-consideration-listing-dart-identification-committee-and

F344 rats exposed to *n*-hexane, the metabolism of MnBK to 2,5-HD proceeded rapidly, while further metabolism of 2,5-HD and its elimination proceeded more slowly (Bus *et al.*, 1981).

As shown in Figure 1 below, *n*-hexane is metabolized by hepatic mixed function oxidases to 2-hexanol. 2-Hexanol can be either oxidized to MnBK, or metabolized to 2,5-hexanediol via ω-1 oxidation. Both 2,5-hexanediol and MnBK can be oxidized to form 5H2H. 5H2H can be oxidized to form 2,5-HD.

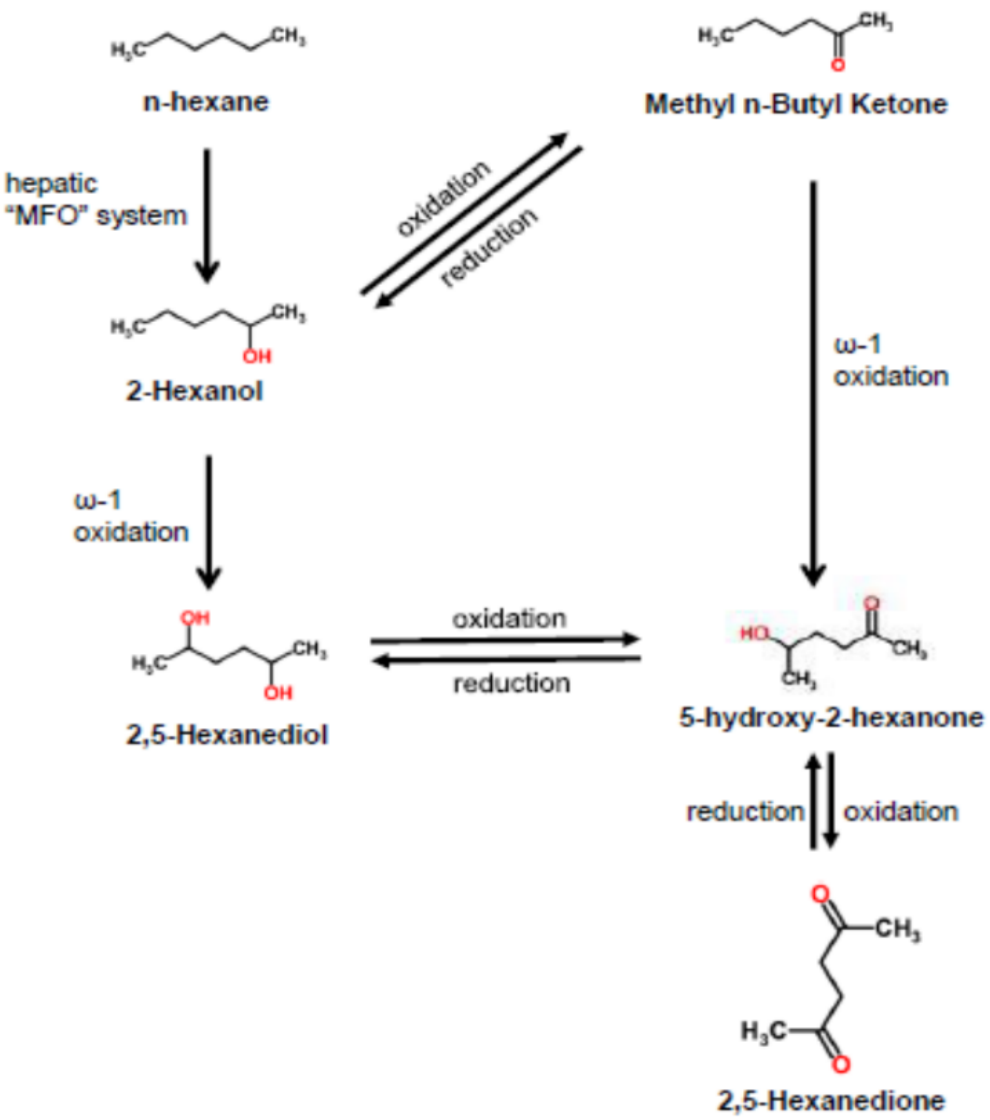


Figure 1. Schematic metabolic pathway for *n*-Hexane and MnBK, modified from Krasavage et al., 1980

Relevant Studies on the Reproductive Toxicity of n-Hexane

No studies in OEHHA's literature search were identified regarding reproductive effects in humans after exposure to *n*-hexane. OEHHA notes that the animal toxicology study by Lui et al. (2013) refers to two reports of reproductive effects in humans, i.e., one meeting abstract and one Chinese-language case report.

Ten studies on developmental toxicity, four studies on female reproductive toxicity, and seven studies on male reproductive toxicity of *n*-hexane in animal models were identified. (Bus *et al.*, 1979; Litton Bionetics Inc, 1979; Litton Bionetics Inc, 1980; Marks *et al.*, 1980; De Martino *et al.*, 1987; Mast, 1987; Mast *et al.*, 1988a; Mast *et al.*, 1988b; Mast *et al.*, 1988c; Nylen *et al.*, 1989; Stoltenburg-Didinger *et al.*, 1990; Stoltenburg-Didinger, 1991; Linder *et al.*, 1992; Imai and Omoto, 1999; Liu *et al.*, 2012; Liu *et al.*, 2013; Li *et al.*, 2014; Li *et al.*, 2015)

Study design parameters and findings of each of these studies on *n*-hexane are summarized in the following tables:

Table 1. *n*-Hexane: Studies Reporting on Developmental Effects
Table 2. *n*-Hexane: Studies Reporting on Female Reproductive Effects
Table 3. *n*-Hexane: Studies Reporting on Male Reproductive Effects

Citations for the tabulated studies are provided in the References section following the tables.

As noted above, studies of the reproductive toxicity of metabolites of n-hexane are also relevant to the consideration of its reproductive toxicity, and are tabulated in Attachment I.

Table 1. *n*-Hexane: Studies Reporting on Developmental Effects

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parental	Developmental	
Bus et al., 1979	*n*-Hexane Phillips Chemical Co., Bartlesville, OK, USA 99.0% pure	Fischer 344 rats Timed pregnant females Gestation day (GD) 8-12 N = 7/group GD12-16 Control =6 Hexane=9 GD 8-16 Control=3 Hexane=8	<u>Perinatal Studies</u> Exposed on GD 12, 12-16, or 8-16. Sacrificed on GD 22. Developmental effects (mortality, number, position and weight) of fetuses noted after GD12-16 and pups allowed to deliver. Culled to 6 pups/litter and weekly mortality recorded. Weaned at 4 weeks.	Inhalation on GD 8-12, 12-16, or 8-16. 6 h/day Control: Room air	0, 1000 ppm.	Fetal resorptions, fetal body weight, external defects, skeletal anomalies, and visceral soft tissue defects. Postnatal growth (weights) at weekly intervals up to 7 weeks.	*n*-Hexane rapidly and extensively metabolized to MnBK and 2,5-HD. Concentrations of *n*-hexane and metabolites in maternal blood similar to fetal levels.	Authors reported no teratogenic effects. No significant alterations in fetal resorptions, body weights, visible anomalies, soft tissue and skeletal anomalies compared to controls. $p<0.05$) A low incidence of pyelectasia (enlarged renal pelvis) noted in each of the three treatment groups (only in litters containing fewer than three fetuses). Exposure on GD 8-16 resulted in a significant ↓ in postnatal growth rate of the pups (13.9% less than control), most apparent up to 3 weeks after birth; corrected by 7 weeks after birth.	Authors commented that repeated exposures to *n*-hexane apparently prevented the metabolism of *n*-hexane to 2,5-HD and/or enhanced the excretion of 2,5-HD and that it is unclear whether this played a role in the outcome of the perinatal toxicity of *n*-hexane Gestation day (GD) 1= Day vaginal plug found

Bus et al., 1979 (continued)		Animal model as above Number of animals not specified	<u>Disposition Studies</u> Maternal blood, liver, kidney, brain and whole fetus (3 fetuses/litter on GD 20 and entire litter on GD 12) analyzed for presence of *n*-hexane, MnBK, and 2,5-HD	Inhalation (6 hours) GD 12 or GD 20 or GD 15-18	1000 ppm	Disposition of *n*-hexane, MnBK and 2,5 HD in maternal tissues (liver, kidney, brain, blood) and fetus.	*n*-Hexane rapidly metabolized to MnBK and 2,5-HD (in all the tissues examined). At 8 hrs minimal or nondetectable levels of *n*-hexane noted. In contrast, tissue levels of 2,5-HD ↑ between 0 and 4 hr and exhibited a slower elimination rate compared to *n*-hexane and MnBK..	After GD 12 exposure: similar maternal blood and fetal levels of 2,5-HD (2.94 + 0.16 pg/ml and 2.49 + 0.17 pg/g wet wt respectively). At 0 hours - levels of 2,5 HD ↑ in maternal blood and fetal tissues more after GD 15-18 exposure than after exposure on GD 20 At 4 and 8 hours - levels of 2,5-HD were ↓ more after GD 15-18 than after exposure on GD 20	Fetal concentrations of *n*-hexane and its metabolites similar to those in maternal blood at all times after exposure.

Reference	Experimental Parameters						Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations	Endpoints Assessed	Parental	Developmental	
Litton Bionetics, 1979	*n*-Hexane Fisher-Scientific Company	Sprague-Dawley rats N= 20/group	Teratology study	Inhalation on GD 6-15 6 h/day	0, 93.4, 408.7 ppm	Mated female rats were weighed on GD 0, 6, 15 and 20 Food consumption measured during the periods GD 0-6, 6-15 and 15-20. Female rats observed daily for changes in general appearance, behavior and condition. At sacrifice on GD 20: Implantation sites, live and dead fetuses, resorption sites, fetal weights, external morphology, soft tissue and skeletal evaluations	No adverse effects in the dams	No induced terata, variation in sex ratio, embryo toxicity or inhibition of fetal growth and development. ↓ Litters with resorption (not statistically significant): 60% in controls and 50% and 41% in 93.4 ppm and 408.7 ppm groups, respectively. Authors reported no significant difference in skeletal effects seen between groups. Authors reported no soft tissue/visceral abnormalities in any group (details not provided). According to authors, skeletal effects observed were related to retarded bone ossification and not malformations as such	

Reference	Experimental Parameters						Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations	Endpoints Assessed	Parental	Developmental	
Marks et al., 1980	*n*-Hexane Fisher Scientific Co. 99% pure	CD-1 Mice (Outbred) male and nulliparous female 60-90 days old <u>Once a day</u> N= 6-14 Controls=37	Developmental toxicity study Dams sacrificed on GD18	Oral gavage GD 6-15 Once a day Vehicle: Cottonseed oil	0 (vehicle), 0.26, 0.66, 1.32, 2.20 g/kg/day	Dam body weights on GD 1, GD 6-15 and GD 18. Fetuses examined for external malformations, skeletal defects and visceral alterations.	Maternal toxicity at 2.20 g/kg/day (1 of 14 dams died) with significant decrease in weight gain	No significant increase in malformations.	Typographical errors in report. GD 1= Day vaginal plug found
		CD-1 Mice (Outbred) male and nulliparous female 60-90 days old <u>3 times/day</u> N= 24-33 Controls=24	As above	Oral gavage GD 6-15 <u>3 times/day</u> 9.00 am 12.00 noon 3.00 pm Vehicle: Cottonseed oil	<u>3 times/day</u> 0 (vehicle), 2.17, 2.83, 7.92, 9.90 g/kg/day (total daily dose)	Dam body weights on GD 1, GD 6-15 and GD 18. Fetuses examined for external malformations, skeletal defects and visceral alterations	Some lethality in dams: 2/25 at 2.83, 3/35 at 7.92, and 5/33 at 9.90 g/kg/day No effect reported on body weight gain	↓ fetal weights at 7.92 and 9.90 g/kg/day. No significant increase in malformations $p<0.05$	Typographical errors in report. GD 1= Day vaginal plug found

Reference	Experimental Parameters						Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations	Endpoints Assessed	Parental	Developmental	
Litton Bionetics, 1980	*n*-Hexane Source and purity not stated	CD-1 male mice. 11 weeks old N=12 males/time/group Females=2/male	Dominant lethal Females were sacrificed at: Week 1, N=22 (100 ppm) and 21 (400 ppm) Week 2, N=16 (100 ppm) and N=23 (400 ppm)	Inhalation (males only) 6 h/day, 5 days/wk, for eight wks	100 or 400 ppm. Negative control: filtered air Positive control: injected once *ip* with triethylene melamine (TEM) at 0.3 mg/kg.	Six parameters were evaluated in this assay: 1. Fertility indices of females at about 14 days from mating. 2. Number of implantations. 3. Number of resorptions 4. Number of dead implants 5.Proportions of females with two or more dead implants 6. Dead implants/live implants ratios	Not available	The high dose shows a significant reduction in the average number of dead implants per pregnant female in week 1 and a slight but not statistically significant increase in week 2	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parental	Developmental	
Mast, 1987	*n*-Hexane Research Triangle Institute (RTI) lot no. H-201 99.9% purity	Sprague-Dawley rats Timed pregnant females N = 30/group	Developmental toxicity study Pregnant females sacrificed for evaluation on GD 20	Inhalation on GD 6-19 20 h/day Control: Filtered air	0, 200, 1000, 5000 ppm.	Pregnant females weighed on GD 0, 6, 13, and 20. At sacrifice: Implantation sites, placental weights, fetal weights, fetal sex, external morphology, visceral and skeletal evaluations.	Significant decrease in maternal body wt at 5000 ppm on GD 13 and 20 ($p<0.05$ for both).	No significant effect on incidence of intrauterine death and incidence of fetal malformations. ↓uterine weight at 5000 ppm ($p<0.05$). ↓ placental weights for male pups at 1000 ($p<0.05$) and both sexes at 5000 ppm ($p<0.01$) Significant ↓ in fetal weights at 1000 ($p<0.05$) and 5000 ppm ($p<0.01$). Significant ↑ in litter frequency of reduced ossification of sternebrae 1-4 at 5000 ppm ($p<0.01$).	Random assignment to test groups. All fetal outcomes analyzed on a per litter basis. Apparent typographical error in report gives concentration of *n*-hexane as 299% in one location of report

Reference	Experimental Parameters						Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations	Endpoints Assessed	Parental	Developmental	
Mast et al., 1988a	*n*-Hexane RTI lot no. H-222 99.2% purity	Swiss CD-1 mice Timed pregnant females N = 35/group	Developmental toxicity study Pregnant females sacrificed for evaluation on GD 18.	Inhalation on GD 6-17 20 h/day Control: Filtered air	0, 200, 1000, 5000 ppm.	Pregnant females weighed on GD 0, 6, 9, 12 and 18. At sacrifice: Implantation sites, placental weights, fetal weights, fetal sex, external morphology, visceral and skeletal evaluations.	Significant decrease in body weight at 5000 ppm on GD18 ($p<0.05$); significant trend for decreasing body weight with increasing dose ($p<0.05$).	No significant effect on pregnancy rate. Decreased uterine weights at 200 ppm ($p<0.05$) and 5000 ppm ($p<0.01$). Decreased frequency of live fetuses/litter at 5000 ppm ($p<0.05$). Increased frequency of resorptions/litter at all groups but statistically significant only at 200 ppm ($p<0.05$). Significant correlations between increasing exposure concentrations and decreasing live fetuses/litter, and with increasing late resorptions/litter ($p<0.05$ for both). Weights of female fetuses significantly reduced at 5000 ppm ($p<0.05$), and values linearly correlated with increasing exposure concentration ($p<0.05$). No significant effect on weights of males, or males and females combined. Significant ↑ in litter frequency of exencephaly at 5000 ppm ($p<0.05$).	Random assignment to test groups. All fetal outcomes analyzed on a per litter basis. The statistically significant increase in the frequency of exencephaly in the 5000 ppm group was considered to be a spurious result due to an extremely low background frequency of this malformation. Apparent typographical error in report gives concentration of *n*-hexane as 299% in one location of the report

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parental	Developmental	
Mast et al., 1988b	*n*-Hexane Phillips Chemical Company (received from RTI, P.O. Box 12194, Research Triangle Park, NC, USA) Purity: 99.1%	CD-1 male mice. N=30 males/group Two 9-11 weeks old females/male	Dominant lethal	Inhalation (males only) 20 h/day for 5 consecutive days Control: Filtered air.	0, 200, 1000, 5000 ppm (only males)	Testes and epididymis evaluation of germinal epithelium Reproductive status of females 12 days after mating: number and viability of the implants	No evidence of *n*-hexane toxicity was observed in the males	No significant alterations in the reproductive indices	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parental	Developmental	
Stoltenberg-Didinger et al., 1990 and Stoltenberg-Didinger, 1991	*n*-Hexane Merck, Darmstadt, Germany No. 4367 99% pure	<u>Experiment 1</u> Wistar rats Female N= 20/group	Prenatal only developmental toxicity study. Examination of effects on pregnancy rate and developmental parameters including examination of brains of offspring (morphology and enzyme maturation pattern in cerebellum)	Inhalation 23 h/day for 21 consecutive days (presumably 21 days of gestation) Control: Filtered air.	0, 500 ppm	Pregnancy rate, signs of fore-limb or hind-limb weakness in dams, body weight, brain weight, light microscopy of cerebellar cortex in offspring, Enzyme histochemical activity as revealed by formazan deposition was studied (in the primary fissure of the cerebellar vermis at postnatal days [PND] 1, 9 or 21).	In dams no neurological irregularities observed.	Concentration-dependent ↓ birth weight at a comparable litter size and ↓ body weight at PND 9, 17 and PND 25 (more pronounced). Light-microscope images of the fissura prima of the vermis cerebelli showed a delay in migration of the outer granular cells and a persistence of Purkinje cells at a lower stage of development at PND 9. According to the authors, prenatal only exposure to *n*-hexane did not induce a reduction of brain weight in offspring. Delay in histogenesis of the cerebellar cortex at all levels examined Activity of the Purkinje cell apical cones was higher at day 9, reflecting delayed outgrowth of Purkinje cell apical dendritic tree and after day 21, equal formazan deposition in the Purkinje cells with no differences in succinic dehydrogenase	

								(SDH) and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-Tr) activity between prenatally exposed and normal rats (seen either in the external or internal granular cells).	
Stoltenberg-Didinger et al., 1990 and Stoltenberg-Didinger, 1991 (continued)	*n*-Hexane Merck, Darmstadt, Germany No. 4367 99% pure Methyl-ethyl-ketone (MEK) Merck, Darmstadt, Germany No. 6014 99% pure	<u>Experiment 2</u> Wistar rats Female N= 8/group	Prenatal only, and prenatal along with postnatal exposure to either *n*-hexane alone or MEK alone Examination of effects on pregnancy rate and developmental parameters including examination of brains of offspring (morphology and enzyme maturation pattern in cerebellum)	Inhalation Group 1 (Only prenatal): 23 h/day for 21 consecutive days (presumably 21 days of gestation,) Group 2 (Prenatal and postnatal):): 23 h/day for 42 days (total), includes growth spurt of cerebellum(presumed to be 21 days of gestation, and PND 1-21, but not specified) Control: Filtered air.	*n*-Hexane 0, 800 ppm MEK 800 ppm	Pregnancy rate, signs of fore-limb or hind-limb weakness in dams, body weight, brain weight, light microscopy of cerebellar cortex in offspring. Enzyme histochemical activity as revealed by formazan deposition was studied (in the primary fissure of the cerebellar vermis at PND 1, 9 or 21).	No hind leg weakness or paralysis prior to birth of the young in either exposed group. Dams only exposed during gestation (Group 1) remained neurologically normal during the nursing period. Dams exposed after birth to either solvent (Group 2) showed paralytic symptoms in the form of a marked hind limb weakness.	More pronounced ↓ in body weight with pre- and postnatal exposure. Authors do not clearly state which treatment group was more severely affected, but imply that the *n*-hexane group was affected in this experiment.. Newborn animals exposed to *n*-hexane considerably smaller and retarded in development; less active and showed fur irregularities. Purkinje cells of *n*-hexane exposed rats showed a higher SDH and NADH-Tr activity at day 9. A persisting apical cone and delayed formation of the apical dendritic tree of the Purkinje cells in the cerebellum from a 9-day-old *n*-hexane exposed rat. Postnatal exposure to *n*-hexane aggravated the developmental delay.	According to authors, the young rats could be protected from toxic effects of the metabolites of *n*-hexane and MEK because of incomplete metabolism in the immature liver.

Stoltenberg-Didinger et al., 1990 and Stoltenberg-Didinger, 1991 (continued)	*n*-Hexane Merck, Darmstadt, Germany No. 4367 99% pure MEK Merck, Darmstadt, Germany No. 6014 99% pure Mixture of *n*-hexane and MEK (1200 ppm *n*-hexane:300 ppm MEK)	<u>Experiment 3</u> Wistar rats Female N= 8/group	Prenatal only, and prenatal along with postnatal exposure to *n*-hexane alone, MEK alone, or a mixture of *n*-hexane and MEK Examination of effects on pregnancy rate and developmental parameters including examination of brains of offspring (morphology and enzyme maturation pattern in cerebellum)	Inhalation Group 1 (Only prenatal): 23 h/day for 21 consecutive day (presumably 21 days of gestation) Group 2 (Pre- and postnatal): 23 h/day for 51 days (total), includes growth spurt of cerebellum (presumed to be 21 days of gestation, and PND 1-30, but not specified) Control: Filtered air.	0, l000 ppm *n*-hexane (initially 1500 ppm), l000 ppm MEK (initially 1500 ppm), mixture of 1200 ppm *n*-hexane and 300 ppm MEK	Pregnancy rate, signs of fore-limb or hind-limb weakness in dams, body weight, brain weight, light microscopy of cerebellar cortex in offspring. Enzyme histochemical activity as revealed by formazan deposition was studied (in the primary fissure of the cerebellar vermis at PND 1, 9 or 21).	Similar to Experiment 2. Also, after 6 weeks of continuous exposure to the *n*-hexane /MEK mixture, the mother animals showed complete paresis of the hindlimbs as well as incipient paralysis of the forelimbs.	Decrease in postnatal body weight in all *n*-hexane groups more pronounced in animals exposed to solvent mixture than those exposed to *n*-hexane alone (no statistical analysis reported). ↓ in size of brain structures noted. Delay in histogenesis of the cerebellar cortex at all concentrations examined. Light-microscope images of the fissura prima of the vermis cerebelli showed a delay in migration of the outer granular cells and a persistence of Purkinje cells at a lower stage of development at PND 9. Lesions not seen on day PND 30 with only prenatal exposure, but with postnatal exposure; a thinner molecular layer and persistence of an outer granular layer noted on PND30. Pups had no severe neurological disturbances after exposure to the same concentration for 3 weeks within the uterus and 3 weeks after birth. Postnatal exposure aggravated the developmental delay. Exposure to the mixture of *n*-hexane and MEK resulted in more pronounced retardation of cell maturation. At day 9, the Purkinje cells showed persisting maximal	MEK potentiated *n*-hexane-neurotoxicity.

								perikaryal formazan coloration, indicating a high, concentrated NADH-Tr activity. Difference in width of the molecular layer between pre- and postnatally exposed and normal rats greater because of the retarded apical dendrite formation.	

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	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations	Endpoints Assessed	Parental	Developmental	
Li et al., 2014	*n*-Hexane Sigma Chemical Corp. St. Louis, MO, USA Purity not stated	Wistar rats Adult females weighing 210–230 g Adult males weighing 300–320 g N = 5/group	Reproductive and developmental toxicity study	Inhalation GD 1-20 4 h/day	0, 500, 2500, 12500 ppm (0, 1800, 9000, 45000 mg/m^3)	F1 pups: Number (alive)/litter; sex ratio; body weights; number of follicles/ovary on PND 56; ovarian morphology	Rats in the 12500 ppm group had mental symptoms: irritability and an attack tendency	No malformations were found in any of the living pups; No significant difference in pup body weight In the 12500 ppm group: ↓live pups/litter ↓proportion of secondary follicles ↑proportion of atresic follicles $p<0.05$	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Parental	Developmental	
Li et al., 2015	*n*-Hexane Sigma Chemical Corp. St. Louis, MO, USA Purity not stated	Wistar rats Adult females weighing 210–230 g Adult males weighing 300–320 g N = 6/group	Reproductive and developmental toxicity study	Inhalation GD 1-20 4 h/day	0, 100, 500, 2500, 12500 ppm (0, 360, 1800, 9000, 45000 mg/m^3)	F1: Number (alive)/litter PND 54: Body weights; vaginal opening, clinical signs, ovarian histology, estrous cycle duration Ovarian granulosa cells of the F1 *in vitro* Progesterone (P4) and estradiol (E2) levels Expression of female hormone production genes (Star, Cyp11, Cyp17 and Hsd3b), and steroidogenic enzymes	Not assessed	No significant differences in body weight, vaginal opening status, and ovarian pathology between control and exposed groups ↓number of live pups/litter in the 12,500 ppm group p<0.05 ↑duration of the pro-estrus stage in the 100 and 500 ppm groups; ↑estrus duration in the 500 and 2500 ppm groups ↓diestrus stage in the 12,500 ppm group p<0.05 Hormone levels: ↑P4 in the 100 and 500 ppm groups. P4 levels peaked in the 500 ppm group and then decreased in the 2500 ppm group. ↓P4 in the 12,500 ppm group ↓E2 levels in the 2500 and 12,500 ppm groups p<0.05 Gene expression: ↑ expression levels of Star in the 100 ppm group ↑mRNA of Star, Cyp11a1, and Cyp17a1 in the 500 ppm group	

								↓ all four genes in the 12500 ppm group p<0.05 Steroidogenic enzyme expression: ↑Star, Cyp11, and Cyp17; at 500 ppm ↓ for all four enzymes at 12500 ppm p<0.05	

Table 2. *n*-Hexane: Studies Reporting on Female Reproductive Effects

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Liu et al., 2012	*n*-Hexane Sigma Chemical Corp., St. Louis, MO, USA Purity not stated	ICR mice Female 2 months old N = 10/group	Effect on the gonadal function of adult female mice	Inhalation five weeks 4 h/day, 7days/wk	0, 3.0, 15.1, 75.8 mL/m^3	Estrous cycle; Ovulation rate Morphological identification and estimation of ovarian cell structure Hormone serum concentration: FSH, LH, E2, and P4 Apoptotic granulosa cells	Mice in each treated group appeared quiet to different degrees. 75.8 mL/m^3 ↓activity, depilation, ↓appetite, rhabdomyolysis, ulcers in the abdominal area. One animal in the 75.8 mL/m^3 group died ↓body weight in the 75.8 mL/m^3 group ($p<0.05$)	Abnormal estrous cycle ↓ number of ovulated ova in all treated groups ($p<0.01$) ↑ death rate of ovulated ova ($p<0.05$) in the 15.1 and 75.8 mL/m3 groups ↓number of follicles of all types in the high-dose group ($p<0.05$) ↓mature follicle ratio ($p<0.05$) in the 15.1 mL/m3 group No significant difference in FSH, LH, and E2 serum levels ($p>0.05$) ↓P4 serum levels decreased significantly in all treated groups ($p<0.01$) Ovarian histology: ↑ chromatin condensation in granulosa cells in the 75.8 mL/m^3 group Uniformly dispersed nucleoli within the nucleoplasm of granulosa cells Damaged mitochondria in the 75.8 mL/m^3 group with ruptured	

								internal membranes, damaged lipid droplets, and autophagic vesicles ↑ % apoptosis: granulosa cells of mature follicles in the 15.1 and 75.8 mL/m^3 groups ($p<0.01$); atresic follicles in the 15.1 mL/m^3 ($p<0.01$); corpus luteum of the 15.1 and 75.8 mL/m^3 groups ($p<0.05$; and $p<0.01$ respectively)	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Liu et al., 2013	*n*-Hexane Sigma Chemical Corp., St. Louis, MO, USA 100% pure	ICR mice Females: 56 days old N = 10/dose group Males: 56 days old N = 20 Untreated group for mating	*In vivo* Exposed and control females were super ovulated, mated with unexposed males, and fertilized eggs collected at 24, 48 or 56 h post-pregnancy	inhalation exposure 8 h/day for 7 days	0, 5.7, 22.5, 90.9 mL/m^3.	Number of embryos at 24, 48, and 56 h post mating	Not assessed	↓ Number of embryos ($p<0.01$)	
	As above	ICR mice Females: 21 days old, N = 10/dose group	*In vitro* Immature oocytes with a germinal vesicle (GV) cultured in the absence or presence of *n*-hexane Oocytes were likely isolated from females exposed to 0, 5.7, 22.5, or 90.9 mL/m^3 *n*-hexane for 7 days, starting at 21 days of age)	Culture medium for 24 h for one week	Increasing concentrations of *n*-hexane (concentrations not stated)	<u>Immature oocytes</u>**:** Germinal vesicle break down (GVBD), formation of the first polar body, mitochondrial membrane potentials, apoptosis	Not applicable	GVBD: no effect at 0 h in culture at 24 h: 0 mL/m^3= 57.38%. ↓5.7 mL/m^3= 40.79%; ($p<0.01$) 22.5 mL/m^3= 52.42%, (NS) ↓90.9 mL/m^3= 34.43%; ($p<0.01$) ↓First polar body formation prevented at 90.9 mL/m^3 at both 0 and 24h culture time ($p<0.01$) ↑Cell death rates: 0 h, 0 mL/m^3 = 14.81%, 90.9 mL/m^3= 20.86% 24 h, 0 mL/m^3 =27.49%, 22.5 mL/m^3= 34.8%; 90.9 mL/m^3=58.85% ($p<0.05$) ↓ Mitochondrial membrane potential ($p<0.01$) ↑ Apoptotic or unhealthy oocyte cells ($p<0.05$)	Exposure elements of the study design are not clear for the studies in cultured oocytes

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Li et al., 2014	*n*-Hexane Sigma Chemical Corp., St. Louis, MO, USA Purity not stated	Wistar rats Adult females weighing 210–230 g Adult males weighing 300–320 g N = 5/group	Reproductive and developmental toxicity study	Inhalation GD 1-20 4 h/day	0, 500, 2500, 12500 ppm (1800, 9000, 45000 mg/m^3)	F1 pups: Number (alive)/litter; sex ratio; body weights; number of follicles/ovary on PND 56; ovarian morphology	Rats in the 12500 ppm group had mental symptoms: irritability and an attack tendency	No malformations in any of the living pups; No significant difference in pup body weight In the 12500 ppm group: ↓live pups/litter ↓proportion of secondary follicles ↑proportion of atresic follicles ($p<0.05$)	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Li et al., 2015	*n*-Hexane Sigma Chemical Corp., St. Louis, MO, USA Purity not stated	Wistar rats Adult females weighing 210–230 g Adult males weighing 300–320 g N = 6/group	Reproductive and developmental toxicity study	Inhalation GD 1-20 4 h/day	0, 100, 500, 2500, 12,500 ppm (360, 1800, 9000, 45000 mg/m^3).	F1: Number (alive)/litter PND 54: body weights; vaginal opening, clinical signs, ovarian histology, estrous cycle duration Ovarian granulosa cells of the F1 *in vitro* P4 and E2 levels Expression of female hormone production genes (Star, Cyp11, Cyp17 and Hsd3b), and steroidogenic enzymes	Not assessed	No significant differences in body weight, vaginal opening status, and ovarian pathology between control and exposed groups ↓number of live pups/litter in the 12,500 ppm group ($p<0.05$) ↑duration of the pro-estrus stage in the 100 and 500 ppm groups; ↑estrus duration in the 500 and 2500 ppm groups ↓diestrus stage in the 12,500 ppm group $p<0.05$ Hormone levels: ↑P4 in the 100 and 500 ppm groups. P4 levels peaked in the 500 ppm group and then decreased in the 2500 ppm group. ↓P4 in the 12,500 ppm group ↓E2 levels in the 2500 and 12,500 ppm groups $p<0.05$ Gene expression: ↑ expression levels of Star in the 100 ppm group ↑mRNA of Star, Cyp11a1, and Cyp17a1 in the 500 ppm group ↓ all four genes in the 12500 ppm group $p<0.05$	

								Steroidogenic enzyme expression: ↑Star, Cyp11, and Cyp17; at 500 ppm ↓ for all four enzymes at 12500 ppm $p<0.05$	

Table 3. *n*-Hexane: Studies Reporting on Male Reproductive Effects

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	Comments
De Martino et al., 1987	*n*-Hexane Merck, Darmstadt, Germany Analytical grade, 99% pure	Sprague-Dawley rats Male 180-220g N = 12-30/ group	Male reproductive toxicity study	Inhalation Single 24-h exposure with recovery allowed for 2 to 30 days 2 control groups: One received food and water ad libitum; another group served as the pair-fed control group and received water ad libitum.	0, 5000 ppm	General condition and walking capability observed daily Body weights at weekly intervals (Students t test) Electromyographic data such as motor conduction Velocity (Mann-Whitney U test) Histopathogy of testes and epididymides (2-3 per group)	None reported	50 to 75% of treated animals had morphological lesions of the testes and epididymides. Immediately after 24 h of continuous exposure, some rats showed focal swelling and degeneration of spermatocytes from stage XII to stage III; meiotic metaphase spermatocytes (stage XIV) appeared to be remarkably affected. Effects on primary spermatocytes from leptotene to middle pachitene stages and spermatids at late stages of maturation; also numerous exfoliated, injured germ cells reached the epididymis. Lesions include focal degeneration of spermatocytes and mild exfoliation of elongated spermatids. Observations during the 2-30 day recovery period: Increased damage to the seminiferous epithelium for first 7 days, with focal	Lesions seen after a continuous 24 h exposure are typically reversible however; they are not reversible when lesions have progressed beyond a certain stage. The causal agent of the lesions is most probably 2,5-HD, a chemically reactive metabolite of *n*-hexane.

								infiltration by inflammatory cells in epididymis; recovery from Days 14 to 30.	
De Martino et al., 1987 (continued)	As above	As above	Male reproductive toxicity study 2 control groups: One received food and water *ad libitum*; another group served as the pair-fed control group and received water *ad libitum*.	Inhalation Repeated 16 h/day exposures (daily for 2 to up to 8 days).	0, 5000 ppm	General condition and walking capability observed daily Body weights at weekly intervals (Students t test) Electromyographic data such as motor conduction Velocity (Mann-Whitney U test) Histopathogy of testes and epididymides (2-3 per group)	After 2 days of treatment body growth was slightly impaired. No signs of polyneuropathy Motor conduction velocity ↓ after 1 week of treatment with clinical symptoms of polyneuropathy	Testicular lesions more pronounced than after 24 h treatment. After 8 days there was massive exfoliation of apparently normal and degenerated spermatids and spermatocytes at various stages of differentiation.	
		As above	Male reproductive toxicity study	Inhalation Repeated 16 h/day exposures (6 days/wk for up to 6 weeks) with recovery allowed for 5 to 29 weeks. 2 control groups: One received food and water *ad libitum*; another group served as the pair-fed control group and received water *ad libitum*.	0, 5000 ppm	General condition and walking capability observed daily Body weights at weekly intervals (Students t test) Electromyographic data such as motor conduction Velocity (Mann-Whitney U test) Histopathogy of testes and epididymides (2-3 per group)	Clinical symptoms of polyneuropathy seen in most animals beginning after 4 to 6 weeks of exposure. Average ↓in body weight (20 to 30%) from 1st – 6th week of treatment. Food consumption ↓ by about 30%. Growth curve of pair-fed controls was between those of experimental	Progressive increases in testicular and epididymal lesions, aplasia of germinal epithelium (spermatogonia). Treatment for 2-4 weeks resulted in nuclear vacuolated and/or multinucleated round spermatids and spermatocytes, massive exfoliation and degeneration of spermatids and prophase spermatocytes, ↑ necrotic spermatocytes at metaphase, and ↓ in number of spermatogonia. Sertoli cells showed nuclear swelling and	Wide range of tubular lesions; increases in severity in relation to the length of treatment. Probably effect of 2,5-HD resulting in 'giant axonal degeneration" due to modification of axonal cytoskeletal protein.

De Martino et al., 1987 (continued)							animals and *ad libitum* controls	vacuolization. Numerous degenerated germ cells found in the epididymal tubule. Treatment for 5-6 weeks induced a ↓ in diameter and collapse of the seminiferous tubules and, in some cases, development of tubules containing only Sertoli cells and rare spermatogonia (aplasia). Numerous lipid droplets noted in the cytoplasm of Sertoli cells. Observations during the recovery period: epididymal epithelium showed morphological alterations (hypertrophy with development of cystlike structures and deep invaginations of the lumen) and a large amount of amorphous material coagulated inside the lumen. Testicular damage continued to progress and aplasia noted in most animals. Numerous inflammatory cells seen in the interstitium and inside the epithelium of the caput epididymis. Severe lesions with complete atrophy of seminiferous tubules, leading to irreversible sterility.	Primary target of repeated exposure could be either the Sertoli cells or the germinal epithelium. Recovery from clinical symptoms was not accompanied with a regression of testicular pathology. Pair-fed controls did not show histological alterations of the testis or epididymis.

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Litton Bionetics, 1980	*n*-hexane Source and purity not stated	CD-1 male mice. 11 weeks old N=12 males/time/group Females=2/male	Dominant lethal Females were sacrificed at: Week 1, N=22 (100 ppm) and 21 (400 ppm) Week 2, N=16 (100 ppm) and N=23 (400 ppm)	Inhalation (males only) 6 h/day, 5 days/wk, for eight wks	100 or 400 ppm. Negative control: filtered air Positive control: injected once *ip* with triethylenemelamine (TEM) at 0.3 mg/kg.	Six parameters were evaluated in this assay: 1. Fertility indices of females at about 14 days from mating. 2. Number of implantations. 3. Number of resorptions 4. Number of dead implants 5.Proportions of females with two or more dead implants 6. Dead implants/live implants ratios	Not available	Only the high dose group had a small but statistically significant increase in fertility indices in week 2 compared to the negative control. These results indicate that *n*-hexane does not cause any reduction in the fertility of the treated males. Females mated to males treated at both dose levels showed no significant difference from the negative control females in both weeks 1 and 2 on all endpoints assessed. The high dose shows a significant reduction in the average number of dead implants per pregnant female in week 1 and a slight but not statistically significant increase in week 2	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Mast et al., 1988b	*n*-Hexane Phillips Chemical Company (received from Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC, USA) Purity: 99.1%	CD-1 male mice. N=20 males/group for dominant lethal Two 9-11 weeks old females/male 10 males/group sacrificed after exposure for evaluation of germinal epithelium	Dominant lethal	Inhalation (males only) 20 h/day for 5 consecutive days Control: Filtered air.	0, 200, 1000, 5000 ppm	Testes and epididymis evaluation of germinal epithelium Reproductive status of females 12 days after mating: number and viability of the implants	No evidence of *n*-hexane toxicity was observed in the males	No significant alterations in the reproductive indices.	Evaluation of germinal epithelium not completed because of lack of dominant lethal effects.

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Mast et al., 1988c	*n*-Hexane Phillips Chemical Company Purity: 99.1%	B6C3Fl mice Male	Male reproductive toxicity study	Inhalation 20 h/day for 5 consecutive days 2 positive control groups: 200 or 250 mg/kg ethyl methane sulfonate, a known mutagen, once each day for 5 consecutive days	0, 200, 1000, 5000 ppm	Body weights and gross lesions of the reproductive tract and morphological evaluations of epididymal sperm	No difference in mean body weights between *n*-hexane groups and controls	No significant effects on the morphology of sperm relative to that of the control group. A significant, dose-related ↓ in the percentage of normal sperm 5 weeks post-exposure was demonstrated for the positive control agent, ethyl methane sulfonate.	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Nylen et al., 1989	*n*-Hexane GR Merck Purity not stated (Toluene and xylene from same source, purity also not stated)	Sprague-Dawley rats Males 250-300 g N = 12/group	Testicular and germ cell line morphology study All rats taken for morphology 12 months after exposure ceased	Inhalation 21 h/day, 7 days/wk, for 28 days. Control: ambient air	0, *n*-hexane only (986 ± 55 ppm), toluene only (982 ± 52 ppm), or *n*-hexane plus toluene (988 ± 54 ppm and 996±56 ppm, respectively)	Macroscopical and light microscopical examination of testes and epididymides stained with hematoxylin and eosin for all rats. Bone marrow from the sternum was assessed	Reduced body weight seen in in 4 of 6 animals 2 weeks after exposure and 3 of 6 animals 10 months post-exposure. The muscles of the hind limbs in all rats with testicular changes were severely atrophic. Bone marrow depression was not found in any exposure group.	Severe testicular atrophy involving the seminiferous tubules with loss of the nerve growth factor (NGF) immunoreactive germ cell line was observed in animals exposed to *n*-hexane only. 10 of 11 animals exposed to *n*-hexane had bilateral testicular damage 1 year after exposure.	Toluene and xylene were found to protect from *n*-hexane induced testicular atrophy.

Nylen et al., 1989 (continued)		Sprague-Dawley rats Males 250-300 g N = 18/group	Testicular and germ cell line morphology Six rats/group taken for morphology at 2 weeks, 10 months, and 14 months after exposure ceased	Inhalation 18 h/day, 7 days/wk, for 61 days. Control: ambient air	0, *n*-hexane only (999 ± 29 ppm), xylene only (1009 ± 47 ppm), or *n*-hexane plus xylene (1010 ± 37 ppm and 1008 ± 42 ppm respectively)	Morphology of testes and epididymides Androgen biosynthetic capacity of testis, testosterone blood levels, vas deferens morphology, noradrenaline (NA) levels, epididymal sperm morphology	The muscles of the hind limbs in all rats with testicular changes were severely atrophic. The testes and hind limbs of the remaining *n*-hexane-treated rats appeared normal	4 of 6 animals exposed to *n*-hexane only had bilateral testicular damage and reduced body weight 2 weeks after exposure, and 3 of 6 rats had bilateral testicular damage and reduced body weight 10 months post-exposure. Total loss of the germ cell line in a fraction (50-66%) of animals up to 14 months post-exposure, indicating permanent testicular damage. No impairment of androgen synthesis or androgen dependent accessory organs.	Authors think that simultaneous exposure to xylene or /toluene reduces the blood levels of the metabolite 2,5-HD, thus protecting from the toxic effects of *n*-hexane
		Sprague-Dawley rats Males N = 3 from each exposure group in the *n*-hexane / xylene study above Females 250 g N = 3/treated male	Fertility Study: 13 months after exposure. Randomly selected males mated with 3 normal females for up to 35 days	See the *n*-hexane / xylene study above	See the *n*-hexane / xylene study above.	Fertility: males with no pregnant females were defined as non-fertile.	See above	Two of three rats exposed to *n*-hexane only were fertile. These two rats were later found to have 100% intact spermatozoa. The third animal was not fertile, and had no spermatozoa. All rats exposed to xylene (three rats) or a mixture of *n*-hexane and xylene (three rats), were fertile.	

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Linder et al., 1992	*n*-Hexane Sigma Chemical Co. Purity not stated	Sprague-Dawley Rats Male 90 days old N = 4-6/group	Male reproductive toxicity study Multiple endpoints assessed over a 2.5-week period following 1 day exposure on day 0 and sacrificed on day 2 or 14.	Oral by gavage for *n*-hexane and positive controls (except for Busulfan: intraperitoneal injection)	20000 mg/kg in 20 ml/kg (equal portions at 9 am and 4 pm) Positive Controls: Benomyl 400 mg/kg in corn oil; Busulfan (intraperitoneal) 10 mg/kg in DMSO/water; Ethylene glycol monomethyl ether (EGME) 250 mg/kg in 5 ml/kg corn oil; Nitrobenzene 300 mg/kg in 5 ml/kg corn oil	Body weight, organ weight Sperm counts in testis and epididymis (Caput/Cauda) Sperm morphology and motility Histopathology of testis and epididymis	No systemic effects reported.	Day 2: Decrease in testicular sperm head count per gram of testis No histopathological changes detected. Day 14 Increased weight of seminal vesicles. No histopathological changes detected	A total of 14 compounds were tested, but only results for *n*-hexane are presented

Linder et al., 1992 (continued)	As above	As above	Male reproductive toxicity study Multiple endpoints assessed over a 2.5-week period following 5 days exposure (days 0-4) and sacrificed on day 8 or 17 (3 or 13 days after the last dose)	Oral by gavage for *n*-hexane and positive controls (except for Busulfan: intraperitoneal injection) 5 daily doses	10000 mg/kg in 10 ml/kg corn oil Positive controls same as above	Body weight, organ weight Sperm counts in testis and epididymis (Caput/Cauda) Sperm morphology and motility Histopathology of testis and epididymis	Decreased body weight ($p<0.05$)	Day 3: No histopathological changes detected Decreased prostate weight Day 13: No histopathological changes detected. Increased sperm counts in Cauda ($p<0.05$)	The positive controls were known to cause specific testicular effects and effects seen in this study were consistent with what has been shown earlier.

Reference	Experimental Parameters					Endpoints Assessed	Results (Effects/NOEL/LOEL)		Comments
	Chemical (Source/ Purity/ Preparation)	Animal Model (Species/ Strain/Sex/ Age) N	Study Design	Exposure (Route/Period/ Frequency/ Vehicle)	Doses/ Concen-trations		Systemic Toxicity	Reproductive Toxicity	
Imai and Omoto, 1999	*n*-Hexane Wako Chemical, INC., Japan Special grade, purity not stated	F344/Jcl rats Male 72 days old N = 6/group	Male reproductive toxicity study. Histological examination of various organs and testes after exposure to *n*-hexane.	Inhalation, in metabolic chamber 415 days 4 h/day, 6 days/wk Control: Ambient fresh air	0, 1000 ppm (measured as 983 ± 32 ppm)	Body weight, testes weight, food intake, frequency of 14 cellular associations in seminiferous epithelium, light microscopic histological findings in testes, incidences of Leydig cell hyperplasia and Leydig cell tumors	Body weight, and food intake not significantly differ from the controls	Increased incidences of Leydig cell hyperplasia and Leydig cell tumors occurred in *n*-hexane exposed rats [100% (6/6), and 33.3% (2/6), respectively] compared to 16.7% (1/6) and 0%, respectively, in the controls. Testes weight, the frequency of 14 cellular associations in the seminiferous epithelium and histological findings in testes (light microscopy) did not significantly differ from controls, Early onset of Leydig cell hyperplasia and Leydig cell tumors observed in the *n*-hexane group suggests the testes were damaged by *n*-hexane	According to the authors, Leydig cell tumors from *n*-hexane exposure apparently differ from those in aged male F-344 rats. Long-term exposure to *n*-hexane is potentially tumorigenic in F-344 rat testes.

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Appendix A: Strategy and Parameters Used for Literature Searches on the Reproductive Toxicity of *n*-hexane

A search of the literature on the reproductive and developmental toxicity of *n*-hexane was conducted under contract by the University of California, Berkeley (Charleen Kubota, M.L.I.S.). The goal was to identify peer-reviewed open source and proprietary journal articles, print and digital books, reports and gray literature that potentially reported relevant toxicological and epidemiological information on the reproductive toxicity of *n*-hexane. The search sought to specifically identify all literature relevant to the assessment of evidence on male reproductive, female reproductive and developmental toxicity.

Databases

The literature search utilized the following search platforms/database vendors:
ChemSpider (Royal Society of Chemistry)
MeSH (Medical Subject Headings) (National Library of Medicine)
Developmental and Reproductive Toxicology Database (DART/ETIC) (National Library of Medicine)
EMBASE® (Elsevier)
Environmental Sciences and Pollution Management (Proquest)
PubMed (National Library of Medicine)
National Technical Research Library (NTRL v3.0) (National Technical Information Service)
ReproRisk® System: **REPROTEXT®** Reproductive Hazard Reference**, REPROTOX®** Reproductive Hazard Information, **Shepard's Catalog of Teratogenic Agents**, **TERIS Teratogen Information System (RightAnswer® Knowledge Solutions OnSite™ Applications)**
Scifinder®: CAS (Chemical Abstracts Service)
TOXLINE (National Library of Medicine)
Web of Knowledge: BIOSIS Previews®, Web of Science® (Thomson-Reuters, Inc.)

Search Process

ChemSpider was searched first to gather chemical names, synonyms, CAS registry numbers, MeSH and Chemical Abstracts Service headings for *n*-hexane before searching bibliographic databases. The MeSH database was used to identify relevant subject headings for reproductive and developmental toxicology endpoints. Relevant subject terms were entered into the PubMed Search Builder to execute a PubMed search.

The following is a typical DART chemical search strategy used to search PubMed:

("*chemical name*" [MeSh] OR CAS *registry number*[RN]) AND ("Congenital Abnormalities"[MeSh] OR "Pregnancy Complications"[MeSh] OR "Reproductive Physiological Phenomena"[MeSh] OR "Embryonic and Fetal Development"[MeSH])

In PubMed, MeSH (Medical Subject Headings) terms at the top of hierarchical lists of subject headings are automatically "exploded" in a search to retrieve citations with more specific MeSH terms. For example, the heading "Congenital Abnormalities" includes numerous specific conditions such as spina bifida and congenital heart defects. The broad subject heading "Pregnancy Complications" encompasses multiple conditions or pathological processes associated with pregnancy. Spontaneous abortion and many fetal diseases are listed under this term.

Additional studies not identified in the primary search but cited in sources such as the ATSDR Toxicological Profile have been included. Some of these studies are not available in the general literature

Additional databases listed above were then searched. The search strategies were tailored according to the search features unique to each database. Web of Science, for example, was searched by entering chemical terms and refining the search by applying Web of Science categories Developmental Biology, Toxicology and/or Public, Environmental and Occupational Health. Sometimes other databases not listed here were searched as needed. For example, if there is a known behavioral endpoint linked to chemical exposure, a social science database such as PsycINFO® would be searched.