

# DEVELOPMENTAL AND REPRODUCTIVE TOXICANT IDENTIFICATION COMMITTEE (DARTIC)

## November 2017 Meeting

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

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# Review of 2015 DARTIC Action on Methyl-n-Butyl Ketone (MnBK) and 2,5-Hexanedione (2,5-HD)

## ➤ MnBK:

DARTIC reaffirmed the listing of MnBK as a chemical known to the state to cause reproductive toxicity:

- Male reproductive toxicity
- Developmental toxicity

## ➤ 2,5-HD:

DARTIC listed 2,5-HD as known to the state to cause reproductive toxicity:

- Male reproductive toxicity

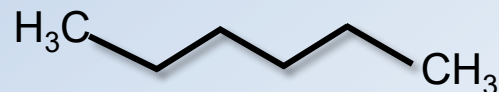
## ➤ *n*-Hexane:

DARTIC requested that OEHHA bring *n*-hexane before the committee



## ***n*-Hexane**

CAS Number: 110-54-3

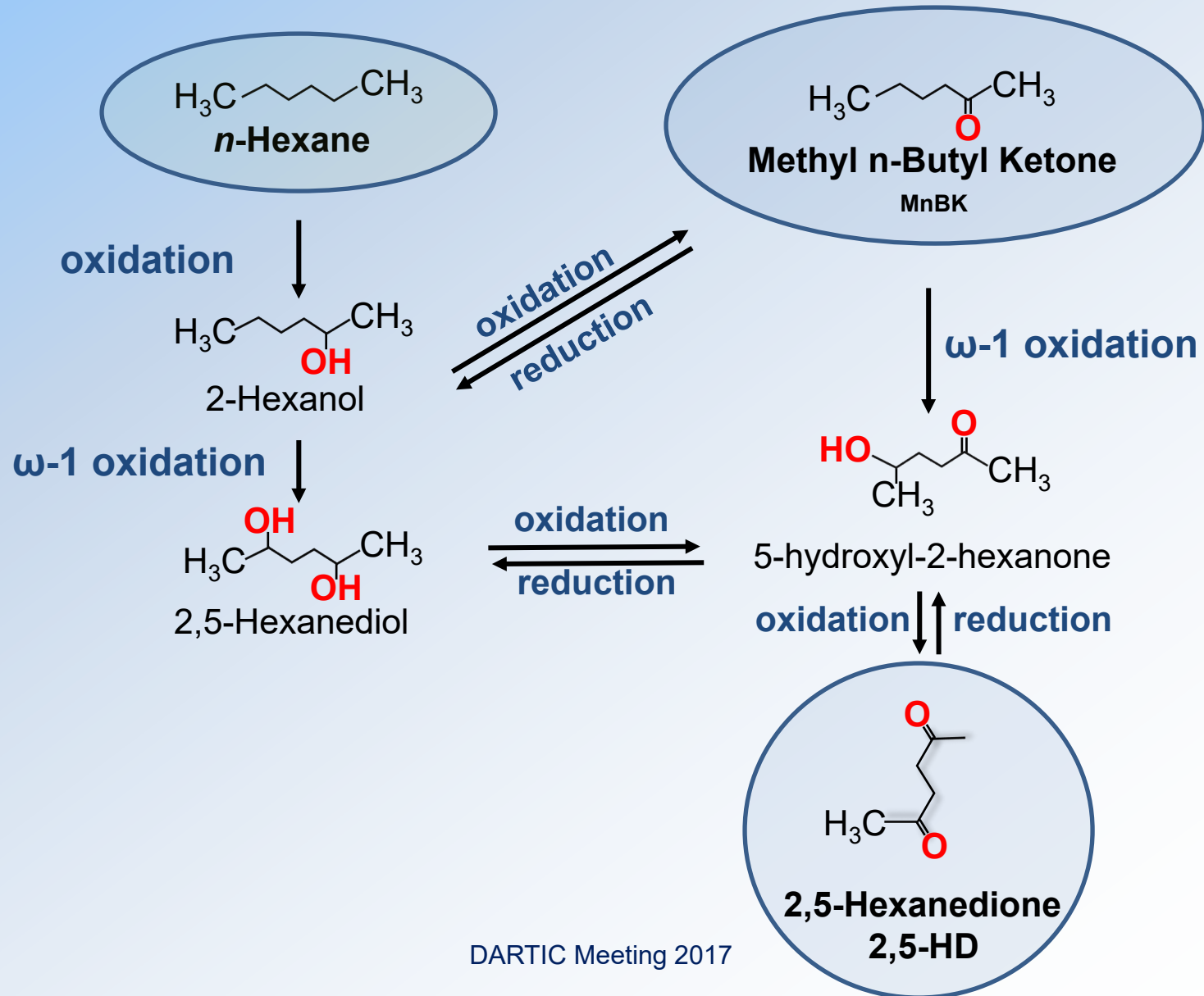


**Uses:** *n*-Hexane is a widely used industrial solvent present in varnishes, cements, glues, and inks. It has also been used as an agent to extract oils from various seeds, including cotton and soybean seeds.



# *n*-Hexane: Metabolic Pathway

(modified from Krasavage et al., 1980)



## Evidence for *n*-Hexane on DART Effects Relevant Studies

- **Ten** studies on developmental toxicity
- **Four** studies on female reproductive toxicity
- **Seven** studies on male reproductive toxicity
- Attachment 1 of the *n*-Hexane hazard identification document (HID) provides relevant studies on DART effects of MnBK and 2,5-HD (2015 HID for MnBK and 2,5-HD)



# *n*-Hexane

## Developmental Toxicity Studies in Rats

Reference	Exposure: Inhalation	Dose (ppm)
Bus et al., 1979	Gestational day (GD) 8-12, 12-16, or 8-16	0, 1000
Litton Bionetics, 1979	GD 6-15	0, 93.4, 408.7
Mast, 1987	GD 8-16	0, 200, 1000, 5000
Stoltenberg-Didinger et al., 1990 and Stoltenberg-Didinger, 1991	GD 1-21	0, 500
	GD 1-21 GD 1-21 + Postnatal day (PND) 1-21	0, 800
	GD 1-21 GD 1-21 + PND 1-30	0, 1000
Li et al., 2014	GD 1-20	0, 500, 2500, 12500
Li et al., 2015	GD 1-20	0, 100, 500, 2500, 12500





# *n*-Hexane

## Developmental Toxicity Studies in Mice

Reference	Exposure	Dose
Marks et al., 1980	<b>Oral by gavage</b> , GD 6-15: Once / day	0, 0.26, 0.66, 1.32, 2.20 g/kg/day
	Three times/day	0 , 2.17, 2.83, 7.92, 9.90 g/kg/day (total daily dose)
Mast et al., 1988a	Inhalation GD 6-17	0, 200, 1000, 5000 ppm
Litton Bionetics, 1980	Inhalation Dominant Lethal 8 weeks exposure	0, 100, 400 ppm
Mast et al., 1988b	Inhalation Dominant Lethal 5 days exposure	0, 200, 1000, 5000 ppm



# *n*-Hexane

## Developmental Toxicity Results in Rats

Reference	Developmental	Maternal
Stoltenberg-Didinger et al., 1990 and 1991	↓ Fetal weight and Birth weight ↓ Postnatal growth rate ↓ Cerebellar development (Postnatal exposure aggravated the developmental delay)	No maternal toxicity reported
Bus et al., 1979	↓ Fetal weight and Birth weight ↓ Postnatal growth rate	
Li et al., 2015	↓ Live pups/litter Abnormal estrous cycle Effects on steroidogenesis	
Litton Bionetics, 1979	None	





# *n*-Hexane Developmental Toxicity Results (continued)

Reference	Developmental	Maternal
Marks et al., 1980 (mice, oral)	<ul style="list-style-type: none"> <li>↓ Fetal weight</li> <li>↓ Birth weight (at the two higher doses)</li> </ul>	<ul style="list-style-type: none"> <li>↑ Dose dependent lethality</li> </ul>
Mast, 1987 (rats)	<ul style="list-style-type: none"> <li>↓ Placental weight (1000 ppm)</li> <li>↓ Fetal weight (1000 ppm)</li> <li>↓ Ossification of sternebrae (5000 ppm)</li> <li>↓ Gravid uterine weight (5000 ppm)</li> </ul>	<ul style="list-style-type: none"> <li>↓ Body weight (5000 ppm)</li> </ul>
Mast et al., 1988a (mice)	<ul style="list-style-type: none"> <li>↓ Gravid uterine weights (200 ppm)</li> <li>↓ Frequency of resorptions/litter (200 ppm)</li> <li>↓ Frequency of live fetuses/litter (5000 ppm)</li> </ul>	<ul style="list-style-type: none"> <li>↓ Body weight (5000 ppm)</li> </ul>
Li et al., 2014 (rats)	<p>At 12500 ppm:</p> <ul style="list-style-type: none"> <li>↓ Live pups/litter</li> <li>↓ Proportion of secondary follicles</li> <li>↑ Proportion of atresic follicles</li> </ul>	<p>Irritability and aggressiveness (12500 ppm)</p>



# Review of Effects of *n*-Hexane, MnBK, and 2,5-HD Developmental Toxicity

<i>n</i> -Hexane	MnBK	2,5-HD
Fetal weight and Birth weight <sup>1,2,3</sup>	Birth weight	Fetal body weight
Live pups/litter <sup>3,4</sup>	Litter size	Embryo lethality
Postnatal growth rate <sup>5</sup>	Postnatal growth rate	---
Cerebellar development <sup>2,3</sup>	Neurological effects in offspring	Neurological effects in offspring

<sup>1</sup> Mast, 1987; <sup>2</sup> Stoltenberg-Didinger et al., 1990 and Stoltenberg-Didinger, 1991; <sup>3</sup> Mast et al., 1988a; <sup>4</sup> Li et al., 2014; <sup>5</sup> Bus et al., 1979



## *n*-Hexane Female Reproductive Toxicity Studies

Reference	Exposure: Inhalation	Dose
Liu et al., 2012	4h/day, 7days/week for 5 weeks	0, 3.0, 15.1, 75.8 mL/m <sup>3</sup>
Liu et al., 2013	8h/day, for 7days	0, 5.7, 22.5, 90.9 mL/m <sup>3</sup>
Li et al., 2014*	GD 1-20	0, 500, 2500, 12500 ppm
Li et al., 2015*	GD 1-20	0, 100, 500, 2500, 12500 ppm

\*Exposure during gestation



## *n*-Hexane: Female Reproductive Toxicity

Reference	Reproductive effects	Systemic Toxicity
Liu et al., 2012 (Mice; 0, 3.0, 15.1, 75.8 mL/m <sup>3</sup> )	Abnormal estrous cycle and ovarian morphology at all doses ↓ P4 at all doses ↑ Apoptosis at 15.1 and 75.8 mL/m <sup>3</sup>	At 75.8 mL/m <sup>3</sup> : Depilation, body weight, rhabdomyolysis, ulcers. One animal died
Liu et al., 2013 (Mice; 0, 5.7, 22.5, 90.9 mL/m <sup>3</sup> )	↓ Number of embryos ↓ First polar body formation ↓ Mitochondrial membrane potential ↑ Apoptotic or unhealthy oocyte ↑ Cell death rates	Not assessed
Li et al., 2014 (Rats; 0, 500, 2500, 12500 ppm)	At 12500 ppm (offspring): ↓ Proportion of secondary follicles ↑ Proportion of atresic follicles	Irritability and aggressiveness (12500 ppm)
Li et al., 2015 (Rats; 0, 100, 500, 2500, 12500 ppm)	Offspring effects: Abnormal estrous cycle Effects on steroidogenesis	Not reported



# Review of Effects of *n*-Hexane, MnBK, and 2,5-HD Female Reproductive Toxicity

<i>n</i> -Hexane	MnBK	2,5-HD
<b>Ovary:</b> Number of embryos First polar body Mitochondrial membrane potential Proportion of secondary follicles and atresic follicles Steroidogenesis, low P4 <sup>1, 2, 3</sup>	---	<b>Ovary:</b> ↓ Protein and DNA content per ovary Fewer medium (growing) oocytes  Litter size at 6 weeks
Apoptosis <sup>1, 2</sup>	---	↓ Cell viability with increased dose and time ↑ Apoptotic index

<sup>1</sup> Liu et al., 2012; <sup>2</sup> Liu et al., 2013; <sup>3</sup> Li et al., 2014



# *n*-Hexane: Male Reproductive Toxicity Studies

Reference	Exposure	Dose (ppm)
Linder et al., 1992	Oral by gavage: 1 day	0, 20000 mg/kg
	5 days	0, 10000 mg/kg
De Martino et al., 1987	Inhalation: Single 24 h exposure Repeated 16 h/day: • for 2 to up to 8 days • for up to 6 weeks	0, 5000 ppm
Mast et al., 1988c	Inhalation: 20 h/day, for 5 days	0, 200, 1000, 5000 ppm
Nylen et al., 1989	Inhalation: 21 h/day, for 28 days 18 h/day, 7 days/week for 61 days.	0, ~1000 ppm
Imai and Omoto, 1999	Inhalation: 4 h/day, 6 days/week for 415 days	0, 1000 ppm
Litton Bionetics, 1980	Inhalation: 6 h/day, 5 days/week, for eight weeks	0, 100, 400 ppm
Mast et al., 1988b	Inhalation: 20 h/day, for 5 days	0, 200, 1000, 5000 ppm



## ***n*-Hexane Male Reproductive Toxicity**

<b>Reference</b>	<b>Reproductive effects</b>	<b>Systemic Toxicity</b>
De Martino et al., 1987	Various degrees of testicular damage	None reported
Mast et al., 1988c	No significant effects on sperm morphology	No body weight effects
Nylen et al., 1989	Severe testicular atrophy Bilateral testicular damage 1 year after exposure	↓ Body weight Atrophic hind limb muscles
Linder et al., 1992	↓ Sperm head count/ gram of testis ↓ Prostate weight ↑ Weight of seminal vesicles No histopathological effects	None reported
Imai and Omoto, 1999	Leydig cell hyperplasia No other histological findings in testes	No body weight effects



## *n*-Hexane Male Reproductive Toxicity (Continued)

<b>Dominant lethal studies in mice</b>		
<b>Reference</b>	<b>Reproductive Effects</b>	<b>Systemic Toxicity</b>
Litton Bionetics, 1980	No male reproductive effect reported	Not available
Mast et al., 1988b	No significant alterations in the reproductive indices	None reported





# Review of Effects of *n*-Hexane, MnBK, and 2,5-HD Male Reproductive Toxicity

<i>n</i> -Hexane	MnBK	2,5-HD
<p>Testicular damage:</p> <p>Severe testicular atrophy Damage persisting 1 year after exposure ↓ Sperm head count/ testis gram<sup>1, 2, 3</sup></p>	<p>↓ Absolute and relative testes weights Atrophy of the testicular germinal epithelium</p>	<p>↓ Testes weight</p> <p>Germ cell depletion Degenerating giant cells: electron-dense cellular debris</p>
<p>↓ Seminiferous tubule diameter</p> <p>↓ Prostate weight ↑ Seminal vesicle weight Leydig cell hyperplasia<sup>1, 3, 4</sup></p>	<p>---</p>	<p>↓ Seminiferous tubule fluid</p> <p>Altered gonadotropins</p>

<sup>1</sup> De Martino et al., 1987; <sup>2</sup> Nylén et al., 1989; <sup>3</sup> Linder et al., 1992; <sup>4</sup> Imai and Omoto, 1999

