November 4, 2013

BY ELECTRONIC MAIL

Ms. Cynthia Oshita
California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
1001 I Street
Sacramento, California 95814

RE: Notice of Availability of Hazard Identification Materials to Support Reconsideration of Listing of 2-Ethylhexanoic Acid (CAS # 149-57-5), September 20, 2013

Dear Ms. Oshita:

The Oxo Process Panel of the American Chemistry Council (ACC) appreciates the opportunity to provide information to the Developmental and Reproductive Toxicant Identification Committee (DARTIC) as it reconsiders the listing of the substance 2-ethylhexanoic acid (2EHA) under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65).[1] ACC's Oxo Process Panel is comprised of companies that produce 2EHA and related substances.[2]

The Office of Environmental Health Hazard Assessment's (OEHHA) September 20 notice indicates that 2EHA no longer meets the criteria for inclusion on Proposition 65 on the basis of the Labor Code mechanism.[3] 2EHA was listed under Proposition 65 on the basis of the Threshold Limit Value (TLV®) developed by the American Conference of Governmental Industrial Hygienists (ACGIH). In March 2012, the federal Hazard Communication Standard (HCS) was amended to remove reference to ACGIH TLVs® as a mandatory basis for establishing that chemicals are hazardous. Consequently, the TLV® for 2EHA no longer provides a basis for listing as a reproductive toxicant under Proposition 65.

OEHHA has identified ten relevant studies on 2EHA for consideration by the DARTIC. A brief analysis of each of these studies is included in Enclosure 1, but the data are summarized below. As outlined, the available data do not support a conclusion that 2EHA has been clearly shown through

---

1 http://www.oehha.ca.gov/prop65/hazard_ident/092013LaborCodeHID.html

2 Oxo Process Panel members include BASF Corporation, The Dow Chemical Company, Celanese Corporation, and the Eastman Chemical Company.

3 Health and Safety Code 25249.8(a)

scientifically valid testing according to generally accepted principles to cause reproductive or developmental toxicity, as required for listing under Proposition 65.

Since there is no published information on the potential reproductive and developmental effects in humans exposed to 2EHA, the DARTIC's assessment must depend on the evidence in rodents. Several animal studies indicate that the developmental effects caused by 2EHA in laboratory tests were observed at doses that resulted in clear evidence of maternal toxicity. Studies suggesting developmental effects in the absence of conventionally defined maternal toxicity are seriously flawed and should not be used as a basis for assessing potential effects of 2EHA. Similarly, evidence for reproductive effects in laboratory animals comes from a study based on fundamentally flawed methodology.

Developmental Toxicity

Although a few studies have observed wavy ribs and delayed ossification in fetal rodents, studies conducted by Taubeneck *et al.* (1994; 1996)[4,5] and Bui *et al.* (1998) demonstrate that the developmental effects caused by 2EHA resulted from a maternally-mediated mechanism of toxicity. These researchers observed that the effects were caused by a deficiency in zinc available to the fetus caused by elevated maternal levels of metallothionein (MT), a zinc-binding protein. The elevated MT levels served to lower zinc levels in the maternal plasma such that delivery of this essential element to the fetus was compromised.

In the most recent of these studies by Bui *et al.* (1998), gavage bolus dosing of 2EHA caused an acute-phase response within the maternal liver, resulting in the induction of MT with subsequent sequestration of zinc within the liver. Binding of maternal zinc by MT within the maternal liver caused decreases in maternal serum zinc levels with corresponding embryonic zinc deficiencies. Bui *et al.* also demonstrated that adequate supplementation of zinc to the dam prevented the developmental effects from occurring. This mechanism of action is not expected to occur in humans due to the lack of significant exposure to 2EHA and the absence of zinc deficiency in the human population.

Although Pennanen *et al.* (1992) observed skeletal abnormalities (wavy ribs, reduced cranial ossification) in Wistar rats exposed to 2EHA at doses that did not produce maternal toxicity, a number of concerns have been raised about the design, conduct and interpretation of this study. The most significant flaws include evidence of decreased water and food consumption, failure to

---

[4] Taubeneck MW et al. Altered maternal zinc metabolism following exposure to diverse developmental toxicants. Reprod Toxicol 8(1):25-40 (1994).

[5] Taubeneck MW et al. Maternal exposure to 2-ethylhexanoic acid (EHXA), 2-ethylhexanol (EHXO) and valproic acid (VPA) results in alterations in maternal and embryonic zinc status. *Teratology* 53(2):88 (1996)

collect relevant data on maternal toxicity, significant inconsistencies in the data presented in the publication, and confusion over how the authors conducted their "per-litter" analysis. As maternal synthesis of MT has been shown to increase in response to feed restriction and stress,[6] the design of the Pennanen *et al.* study likely played a significant role in the developmental toxicity observed by the authors.

The conclusion that 2EHA does not present a risk of developmental toxicity to humans is supported by the work of Hendrickx *et al.* (1993) who found that the substance was not a selective developmental toxicant in New Zealand white rabbits.

**Reproductive Toxicity**

In a separate study, Pennanen *et al.* (1993) reported evidence of reproductive effects, including delayed fertility and reduced litter size, in Wistar rats. In addition to concerns about water consumption and data inconsistencies, several questions have been raised about the sperm parameters reported in the 1993 paper.

A 14% reduction in drinking water was reported in the pregnant high-dose female groups during gestation, although the water consumption data is only reported for the entire gestation period and not at all for the mating and pre-mating periods. Water consumption values during the lactation period were not included in the publication. Since the authors report using body weight of the females to determine the concentration of 2EHA in the drinking water, the normal increased consumption of water during lactation likely led to heroic dose levels of 2EHA during the first week of lactation.

While it is not unusual in reproductive toxicity studies to have 3-4 animals per group that take several estrus cycles to breed (or to have 1-3 animals pre group that are not pregnant) as reported by Pennanen *et al.* (1993) in the 2EHA-exposed groups, the 100% pregnancy rate within the first two cycles in the control group is quite unusual. Given the unusual method of breeding used in this study (keeping the male and female animals together after evidence of mating was obtained), it is difficult to interpret the fertility data reported in the study.

The sperm parameters reported in the 1993 publication include sperm motility and morphology. The authors report a control value of 34.8 percent reported for sperm motility (Table 3 in the paper) which is well below typically reported values for controls of 85 to 96 percent[7] and

---

[6] Kavlock R. and Rogers J. Developmental Toxicology. IN Casarett L *et al.* (Eds.) Toxicology: The Basic Science of Poisons. Fifth Edition, McGraw Hill Publishers, NY, NY. (1996).

[7] Chapin RE and Conner MW. Testicular Histology and Sperm Parameters. IN An Evaluation and Interpretation of Reproductive Endpoints for Human Health Risk Assessment. HESI ILSI Press, Washington, DC. (1998).

below the minimum value of 70 percent considered acceptable for control populations.[8] The authors provide only a two-sentence description of the observations of altered sperm morphology, which has not been reported in other studies following repeated exposure to levels of 2EHA higher than those used in the Pennanen study.

As outlined above, and as described in the enclosed summary of studies identified by OEHHA, the available laboratory animal data do not support a conclusion that 2EHA has been clearly shown through scientifically valid testing according to generally accepted principles to be a reproductive or developmental toxicant. As such, these data do not provide sufficient basis for listing of 2EHA as known to the state to cause reproductive toxicity under Proposition 65.

Sincerely,

*Steve Risotto*

Stephen P. Risotto
Senior Director

Enclosure

[8] Seed J *et al.* Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: A consensus report. *Reprod. Toxicol*. 10:237-244 (1996).

**Relevant Studies on**
**2-Ethylhexanoic Acid Identified by OEHHA**
**For Consideration by the**
**Developmental and Reproductive Toxicant Identification Committee**

**Bui *et al.* (1998)**

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female (6-10/dose) for Experiments 1 and 2; Male for Experiment 3 |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Various |
| **Exposure period** | : | Various |
| **Frequency of treatment** | : | Various |
| **Duration of test** | : | Various |
| **Doses** | : | Various |
| **Control group** | : | Yes |
| **GLP** | | No |

A series of mechanistic studies were conducted to investigate the effects of developmentally toxic doses of 2-ethylhexanoic acid on zinc metabolism in pregnant rats. The results confirm that 2EHA can induce hepatic MT production, decrease maternal serum zinc concentrations, and if the dose is high enough, may prevent adequate zinc from reaching the developing fetus, resulting in developmental effects.

**Collins et al. (1992)**

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | Female (3-22/group) |
| **Strain** | : | SWV and C57BL/6NCrlBR |
| **Route of admin.** | : | Intraperitoneal injection |
| **Exposure period** | : | See methods |
| **Frequency of treatment** | : | Single to multiple doses per day; see methods |
| **Duration of test** | : | See methods |
| **Doses** | : | 403-1037 mg/kg-bw; see methods |
| **Control group** | : | Yes (physiological saline) |
| **GLP** | | No |

The use of a non-standard strain of mouse (SWV) that has genetic defects leading to increased susceptibility to execephaly is problematic and limits the study's value in a hazard characterization process. The route of treatment (ip injection) was not considered to be appropriate because of the

potential direct absorption of the dosing solution on the uterus and conceptus. Also, the route of administration may have confounded the interpretation of the results by circumventing the normal absorption/metabolism/excretion pathway. Control animals received only physiological saline rather than an isosmotic solution without the test substance. No data on maternal toxicity (weight gain, feed consumption, or clinical signs of toxicity) were provided other than mortality. There was no analysis of the dosing solutions.

**Dawson et al. (1991)**

| | | |
|---|---|---|
| **Type** | : | Developmental toxicity |
| **Species** | : | Frog |
| **Sex** | : | Male and female |
| **Strain** | : | *Xenopus laevis* |
| **Route of admin.** | : | Direct addition to late-blastula stage embryos growing in covered glass Petri dishes |
| **Exposure period** | : | 96 hours |
| **Frequency of treatment** | : | 4 times (solutions replaced every 24 hours) |
| **Doses** | : | Specific concentrations not provided |
| **Control group** | : | Yes |
| **GLP** | | No |

The evaluation of 2-ethylhexanoic acid was one of ten studies conducted using short chain aliphatic acids in the first phase of the investigation described in this publication. The study indicated that all ten acids were likely to induce malformations in a similar manner, though it was determined that 2-ethylhexanoic acid was one of the acids that had the lowest incidences of malformed embryos with multiple defects. The studies on 2-ethylhexanoic acid also resulted in some of the lowest test-to-test variation seen for individual acids in the study.

The relevance of this study to mammalian toxicity is unknown. No data were provided on the actual concentrations of 2EHA used in the testing. It was unclear if the material used was 2-ethylhexanoic acid or the Na-salt of 2-ethylhexanoic acid; however, all data were reported as 2-ethylhexanoic acid.

**Hauck et al. (1990)**

| | | |
|---|---|---|
| **Species** | : | Mouse |
| **Sex** | : | Female |
| **Strain** | : | Han:NMRI |
| **Route of admin.** | : | Intraperitoneal injection |
| **Exposure period** | : | Gestation day 7 and/or 8 |
| **Frequency of treatment** | : | 1-4 times/day |

| | | |
|---|---|---|
| **Duration of test** | : | Through gestation day 18 |
| **Doses** | : | 500, 2000 mg/kg-bw/day |
| **Control group** | : | Yes |
| **GLP** | | No |

The route of treatment (ip injection) was not considered to be appropriate because of the potential direct effects of the dosing solution on the uterine muscle. The route of administration also may have confounded the interpretation of the results by circumventing the normal absorption/ metabolism/excretion pathway. Control animals received only physiological saline rather than an isosmotic solution without the test substance.

Author states that Han strain of mouse used demonstrates susceptibility to exencephaly. Study design not in accordance with OECD guidelines: numbers of pregnant females used was below that recommended by OECD; treatment interval during gestation did not include days 6-15; animals were dosed four times per day rather than once per day. No data of maternal toxicity (weight gain, feed consumption, or clinical signs of toxicity) were provided. There was no analysis of the dosing solutions.

The Han NMRI mouse strain demonstrates susceptibility to exencephaly which have confounded the results. No data on maternal toxicity such as weight gain/loss, feed consumption, or clinical signs of toxicity were provided within the results. There was no analysis of the dosing solutions to know whether the correct concentration of 2-EHA was administered.

**Hendrickx et al. (1993)**

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female (25/dose) |
| **Strain** | : | Fischer 344 |
| **Route of admin.** | : | Gavage |
| **Exposure period** | : | Gestation days 6-15 |
| **Frequency of treatment** | : | Once per day |
| **Duration of test** | : | Gestation days 6-21 |
| **Doses** | : | 0, 100, 250, or 500 mg/kg-bw/day |
| **Control group** | : | Yes |
| **GLP** | | Yes |

| | | |
|---|---|---|
| **Species** | : | Rabbit |
| **Sex** | : | Female (15/dose) |
| **Strain** | : | New Zealand White |
| **Route of admin.** | : | Gavage |
| **Exposure period** | : | Gestation days 6-18 |

| | | |
|---|---|---|
| **Frequency of treatment** | : | Once per day |
| **Duration of test** | : | Gestation days 6-29 |
| **Doses** | : | 0, 25, 125, or 250 mg/kg-bw/day |
| **Control group** | : | Yes |
| **GLP** | | Yes |

The researchers observed maternal and fetal toxicity at the highest dose. There was slight fetotoxicity (reduced skeletal ossification) at250 mg/kg. There was no treatment-related increased incidence of malformations at any dose.

In rabbits, the test substance was not developmentally toxic or teratogenic but did cause maternal toxicity.

**Narotsky et al. (1994)**

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female (15-20) |
| **Strain** | : | Sprague-Dawley |
| **Route of admin.** | : | Oral gavage |
| **Exposure period** | : | Gestation days 6-15 |
| **Frequency of treatment** | : | Daily |
| **Duration of test** | : | 10 consecutive treatment days per animal |
| **Doses** | : | 0, 900, or 1200 mg/kg-bw/day |
| **Control group** | : | Yes, corn oil vehicle only |
| **GLP** | | No |

The main focus of study was the developmental toxicity of valproic acid. 2EHA was included in the study as one of 14 related chemicals evaluated using small groups of animals and few dose levels. The doses of 2-ethylhexanoic acid that were used caused developmental effects indicative of effects on the axial skeleton, at levels causing excessive maternal toxicity. The doses that were chosen were either known to cause maternal toxicity or to represent 75% of that level. As a result, the total numbers of dams, litters, and pups available to be evaluated was low. Extensive maternal respiratory effects in the current study may have been related to the means of dose administration, and may have been contributory to lower than expected numbers of pregnancies.

**Pennanen *et al.* (1992)**

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female (20-21/dose) |
| **Strain** | : | Wistar |
| **Route of admin.** | : | Drinking water |
| **Exposure period** | : | Gestation days 6-19 |
| **Frequency of treatment** | : | Daily, *ad libitum* |
| **Duration of test** | : | Gestation days 6-20 |

| | | |
|---|---|---|
| **Doses** | : | 0, 100, 300, or 600 mg/kg-bw/day |
| **Control group** | : | Yes, deionized water |
| **GLP** | | No |

**Pennanen *et al.* (1993)**

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female (5-12/group) |
| **Strain** | : | Wistar |
| **Route of admin.** | : | Gavage |
| **Exposure period** | : | Gestation day 4, 5, 6, or 7 |
| **Frequency of treatment** | : | Once |
| **Duration of test** | : | Through gestation day 10 |
| **Doses** | : | 600 mg/kg-bw |
| **Control group** | : | No |
| **GLP** | | No |

Several concerns have been expressed regarding the design, conduct and interpretation of these two Pennanen studies. Both of the studies administered 2EHA as a sodium salt in drinking water. However, there are no indications that the concentration of 2EHA was verified analytically or what the stability of 2EHA in water is. Indeed, the current draft IUCLID from the EPA for 2EHA does not contain any data indicating the stability of 2EHA in water. The study design employed involved raising the concentration of 2EHA in the drinking water as the animals gained weight, thereby ensuring a (relatively) constant target dosage in mg/kg body weight. However, there are several problems with this study design. First, if there is a taste aversion to consuming the drinking water by the animals, there will be periods of significant dehydration and decrease in feed consumption until the animals thirst mechanism overcomes the aversion to the taste. This will be episodic as the 2EHA concentrations in the drinking water are raised weekly based on the most recent body weight measures. The only way to know if this occurs is to collect more frequent drinking water measurements and this was not done in either of these studies. While this study design is less problematic for repeated exposure and cancer bioassay designs, it is extremely problematic in reproductive and developmental toxicity study designs as maternal toxicity (as water/feed restriction) can affect reproductive and developmental outcomes. For example, maternal hepatic acute phase response protein (metallothionein) synthesis is increased in response to feed restriction and stress.[1] This is especially important for studies conducted with 2EHA, as it has been demonstrated that 2EHA can also induce hepatic metallothionein synthesis and indeed, this plays a significant role in causation of developmental toxicity by this compound (Bui, et al., 1998). It is important to recognize that the role of maternal toxicity is only as good as the endpoints collected to measure that toxicity. Failure to collect the relevant data does not mean that maternal toxicity does not exist, only that the researchers did not collect the necessary information.

Indeed, in the developmental toxicity study, the high dose group pregnant female rats consumed up to 20% less water than the control group, starting on gestation day (GD) 6. However, effects on body

[1] Kavlock R and Rogers R. Developmental Toxicology. In Casarett L *et al.* (Eds). Toxicology: The Basic Science of Poisons. Fifth Edition, McGraw Hill Publishers, NY, NY (1996).

weight and body weight gain were not noted in the females from the high dose group until GD 13 onward, with a 10% decrease in body weight reported. Interestingly, the investigators only started to collect feed consumption measurements at this time, meaning they were unaware of any decreases in feed consumption prior to this time. The terminal body weight of the dams from the high dose group was 115 less than the concurrent controls and the corrected maternal body weight gain in the high dose group was reduced approximately 54%.

There are also concerns in how the authors reported the data. The authors tended to report the data on a per-fetus or per-pup basis. Within the 1992 developmental toxicity publication, they report the "group mean of litter percentages (affected fetuses per litter)". However, the numbers simply do not add up. For example, within Table 2, the number of "affected fetuses" (described as "fetuses with all malformations" – perhaps all fetuses with malformations?) included 4 fetuses from the Control group for a 2% incidence rate within all Control fetuses (4/202). The fetuses with skeletal malformations made up these 4 Control fetuses with no visceral malformations reported (highly unusual). Since the fetuses were either processed for skeletal or visceral exam, skeletal malformation rate ("the percentage of examined fetuses") was reported as "3.8" (approximately double the 2% overall incidence rate – as expected with only half the fetuses examined for skeletal malformations). However, the "group mean of litter percentage" increases from 2% Control overall to 3.8% for the Control skeletal malformation rate. This cannot be if a) all litters contributed fetuses to the skeletal exam (as described in the Methods) and b) the Control overall "group mean of litter percentages" was described as 2%.

Similar problems persist throughout Table 2 when examining the "group mean of litter percentages" for the exposed groups. Clearly the authors meant to convey something other than the data analyzed on a "per litter basis" which is required under the EPA Developmental Toxicity Test and Risk Assessment Guidelines. The authors continued to use the parameter "group mean of litter percentages" in the 1993 reproductive toxicity publication and again it is not possible to reconstruct exactly what that term means or how it was calculated.

The primary concern regarding the conduct of the reproductive toxicity study (1993 publication) pertains to the water consumption data and the sperm parameters reported in the paper. Nonpregnant female rats consume about 28 mls of water per day. During gestation, water consumption rises very rapidly to approximately 40 mls of water per day (in a recent study, this level was attained by GD 4). During lactation, the amount of water a lactating female rat will drink per day rises to about 58 mls per day. The body weight of the females increases during gestation as well and the authors must have increased the concentration of 2EHA in water to meet the objective mg/kg dose levels. A 14% reduction in drinking water was reported in the pregnant high-dose female groups during gestation, although the water consumption data is only reported for the entire gestation period and not at all for the mating and pre-mating periods. Water consumption values during the lactation period were not included in the publication. The authors would have used the body weight of the females on PND 0 to determine the concentration of 2EHA in the drinking water on PND 0, yet the dams would have been consuming twice as much water per day (58 mls vs. 28 mls) during this time, leading to heroic dose levels of 2EHA during the first week of lactation.

The sperm parameters included within the 1993 publication are problematic for several reasons. The endpoints considered affected by the study authors included sperm motility and morphology. The control values reported for sperm motility (Table 3 in the paper) was 34.8%. As discussed by Chapin and Conner (1998), control values for laboratory rats typically are between 85 to 96% motility.[2] As Seed et al. (1996)[3] pointed out, a minimum value of 70% motile is acceptable in the control population. Something is clearly wrong within the Pennanen laboratory with either the male rat population (in which case they should not have been used for this study) or the method of determining sperm motility within this laboratory (in which case the results of this paper should be discarded as unreliable).

The problem may have been the use of glass slides with cover slips versus a chamber to determine sperm motility. As recommended by Chapin and Conner (1998), a chamber of greater than 20 µm is preferable for rodents, otherwise sperm motility is inhibited. The Pennanen laboratory reported using a "prewarmed glass slide covered with a glass coverslip".

The question of altered sperm morphology is more difficult to address since the authors only provide a two sentence description of how it was done. One point made by the authors in the Pennanen paper is no longer credible. Altered sperm morphology is no longer believed to reflect genetic damage to the sperm (Chapin and Conner, 1998). It has been noted that the morphologic assessment is extremely subjective and that the training and the experience of the observer is of paramount importance.[4] The only parameter reported as changed was an increased percentage of "abnormal heads" (Table 4 of Pennanen, et al., 1993) without a further description of what constituted "abnormal". The other parameter included in Table 4 was "agglutinated sperm" and can easily be due to preparation and handling of the sperm sample prior to microscopic observation. The authors only describe their methodology as "A 1.0 ml portion of the sperm suspension was incubated with 50 l of 1% eosin Y for 45 minutes…" followed by microscopic exam.

It is important to note that abnormally shaped sperm can also be observed during microscopic examinations of sections of the testes and epididymides and yet this has never been reported following repeated exposure to levels of 2EHA higher than those used in the Pennanen study. Given the lack of reproducible findings across studies with repeated exposure of male rats to 2EHA, the increased percentage of altered sperm morphology should be viewed with a healthy dose of skepticism.

---

[2] Chapin RE and Conner MW. Testicular Histology and Sperm Parameters. In An Evaluation and Interpretation of Reproductive Endpoints for Human Health Risk Assesssment. HESI, ILSI Press, Washington, DC (1998).

[3] Seed J *et al.* Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: A consensus report. *Reprod Toxicol* 10:237-244 (1996).

[4] Holson JF *et al.* Significance, reliability, and interpretation of developmental and reproductive toxicity study findings In Developmental and Reproductive Toxicology: A Practical Approach. Second Edition. Taylor & Francis, CRC Press, NY, NY (2006).

**Ritter et al. (1987)**

| | | |
|---|---|---|
| **Species** | : | Rat |
| **Sex** | : | Female (group size/dose not indicated) |
| **Strain** | : | Wistar |
| **Route of admin.** | : | Gavage |
| **Exposure period** | : | Gestation day 12 |
| **Frequency of treatment** | : | Once |
| **Duration of test** | : | Gestation days 12-20 |
| **Doses** | : | 0, 1.0, or 2.0 mL/kg undiluted 2-ethylhexanoic acid (approximately 900 or 1800 mg/kg-bw based on a specific gravity value of 0.9031) |
| **Control group** | : | Yes |
| **GLP** | | No |

The high dose produced embryo- and fetal-toxicity based on the 30% decrease in fetal weight, and 34% increase in percentage dead and resorbed fetuses (from 9.6 in controls to 12.9 in the high-dose). The percentage of malformed fetuses was 0% in control animals and 67.8% in the high-dose dams. No apparent toxic or teratogenic effect was observed at the low dose. Defects observed included hydronephrosis, levocardia, septal defects, short and kinky tail, ectrodactyly, misplaced digits, and bowed radius.

The test substance was developmentally toxic and teratogenic at the high dose. This study did not establish a definitive, valid NOAEL/LOAEL for developmental effects because the test substance was not administered throughout the period of major organogenesis.

Test material purity was not reported. A rationale was not provided for administering only a single dose on GD 12. Total numbers of treated animals or original numbers of pregnant females in each treatment group were not provided. Maternal effects were not described. No statistical analyses were conducted, the number of animals per group was low (only 7), and fetal data are presented as percentages of affected fetuses per litter. Thus, one or two litters could have adversely affected the data.

**Svechnikova *et al.* (2007)**

The authors investigate the effects of di-(2-ethylhexyl) phthalate, the parent compound of 2-EHA, on the hypothalamic- pituitary-gonadal axis of immature female rats as well as *ex vivo* steroidogenesis by granulosa cells and secretion of luteinizing hormone by gonadotropes. Only one *in vitro* experiment incorporates 2-EHA, the metabolite of DEHP, as a test article. In this experiment, 2-EHA is administered with GnRH and the production of LH is measure from primary cultures of pituitary cells from immature rats.

The data collected from a single *in vitro* experiment does not provide sufficient evidence that 2-EHA is a reproductive toxicant. Therefore, this article does not directly relate to the reproductive toxicity of 2-EHA and therefore should not be considered.