

**Review of Scientific Data on
Di-isononyl Phthalate (DINP) and
Di-isodecyl phthalate (DIDP) demonstrating
that neither are endocrine disrupters**

(CAS No. 68515-48-0 / EINECS No. 271-090-9, 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich; CAS No. 28553-12-0 / EINECS No. 249-079-5 di-isononyl phthalate; and CAS No. 68515-49-1 / EINECS No. 271-091-4 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich, CAS No. 26761-40-0 / EINECS No. 247-977-1 di-isodecyl phthalate)

European Council for Plasticisers and Intermediates
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Summary Conclusions

- **DINP and DIDP are not endocrine disrupters**
- **DINP and DIDP are two of the most widely studied and evaluated chemical substances in the world**
 - Studies and evaluations have been conducted intensively and thoroughly over the last 30 years, with demonstrated safe use for 50 years.
- **DINP and DIDP are not endocrine disrupters as defined by Weybridge, IPCS and the draft REACH Guidance**
 - The definitions for endocrine effects (Weybridge definition, International Programme for Chemical Safety [IPCS], draft REACH Guidance) require evidence of adverse health effects in intact organisms, or progeny, or subpopulations mediated via an effect on functioning of the endocrine system.
 - DINP and DIDP have shown no evidence of endocrine related adverse health effects in intact organisms, chronic and sub-chronic toxicology studies, and endocrine screening studies.
 - DINP and DIDP have been found not to cause adverse effects on reproduction in two-generation rodent studies. OECD considers the two-generation study to be the most rigorous for testing and assessing effects of endocrine-disrupting chemicals on the reproductive system.
 - Limited human epidemiological studies also have been conducted on the association of endocrine effects and phthalate metabolite levels in breast milk and urine. These studies, as well, do not show any evidence of an association between DINP and DIDP exposure and endocrine disruption.
- **The EU Risk Assessments for DINP and DIDP evaluated all of the above studies and concluded that there is little concern with regard to potential endocrine effects.**
 - DINP and DIDP have been assessed by the relevant EU authorities for classification and labeling for reproductive effects, with the conclusion that classification and labeling for such effects is not required.
 - In addition the EU authorities have concluded that DINP and DIDP are not considered hazardous under any of the other EU classification categories.
- **For these reasons DINP and DIDP do not meet internationally accepted definitions for endocrine disruption, including those in the REACH Guidance**

DINP and DIDP are not Endocrine Disrupters

The main body of the paper is divided into eight sections:

1. Two generation reproductive studies on DINP and DIDP and the EU Risk Assessment conclusions
2. Status of endocrine testing guidelines
3. Lack of oestrogenic activity for DINP and DIDP
4. Studies on anti-androgenic effects
5. Human data on endocrine effects and DINP/DIDP
6. Definitions for endocrine disrupters
7. Conclusions
8. References

Appendix – The endocrine system

1. Two generation reproductive studies on DINP and DIDP and the EU Risk Assessment Conclusions

DINP and DIDP do not cause reproductive effects in rodent two generation reproductive toxicity studies. These studies provide a comprehensive basis on which to evaluate reproductive and developmental effects, including anti-androgenic effects in male rat pups. As such, DINP and DIDP were not classified as reproductive toxicants as part of the EU Risk Assessments. There are reports that DINP minimally modulated the androgenic endocrine system in developing rats (Gray et al, 2000), but only at doses that are well above relevant exposures, and this modulation did not produce adverse effects. These studies were considered in the recently published EU risk assessments for DINP and it was concluded that the effects are of little concern. The EU Scientific Committee for Toxicology, Ecotoxicology and the Environment (CSTEE) evaluated the endocrine disrupting properties of DINP to be “very low”; CSTEE took into account the slight effects seen at very high dose levels.

2. Status of endocrine testing guidelines

There is currently much effort focused on the development of validated in vivo and in vitro test methods for identifying endocrine disrupting substances notably under the OECD Test Guidelines programme, and in the United States and Japan. However, there are currently no internationally agreed methodologies or criteria available for the evaluation and confirmation of endocrine effects. OECD has developed a conceptual framework for the testing and assessment of potential endocrine disrupters, and included at the highest level (Level 5) is the 2-generation reproductive study. Inclusion at Level 5 underlines the importance of this test in defining endocrine disrupters.

3. Lack of oestrogenic activity for DINP and DIDP

Initially, discussion of endocrine modulation and phthalate esters focused on oestrogenic effects. Some phthalate esters were found to bind to the oestrogen receptor; however,

the binding was later determined to be non specific and did not lead to an activation of the oestrogen receptor. Lack of oestrogenic activity of phthalates was confirmed in cellular and whole animal assays for oestrogenic activity. In this analysis, both DINP and DIDP were evaluated and found to be without effect. As such, it is now accepted in the scientific community that DINP and DIDP are not oestrogenic nor anti-oestrogenic (Zacharewski et al., 1999).

4. Studies on anti-androgenic effects

Some oral gavage studies on DINP (Gray et al., 2000; Borch et al., 2004) have shown effects in male rat offspring at a single very high dose level, which is well above relevant exposure levels. The effects produced were of low incidence (observed in few pups) and were of low severity (reduced testosterone synthesis, areola or nipple retention). It is possible that these effects are specific to rats e.g. nipple retention is normal in humans but not normal in male rats. Further research is ongoing to understand the mechanism of these effects and whether they are rodent specific. It should be noted that the two generation reproductive toxicity study is the definitive study in this respect and no reproductive effects were seen in the DINP two-generation study (Waterman et al., 2000). To date, no data are available that indicate DIDP produces anti-androgenic effects in male rats, and no reproductive deficits were observed in two generation studies in rats with DIDP.

5. Human data on endocrine effects and DINP/DIDP

Recent research studies have evaluated relationships between fetal and neonatal exposure to phthalates and markers of endocrine mediated reproductive toxicity in humans. Swan et al (2005) investigated the association between metabolites of several phthalates in urine and anti-androgenic effects in young boys. Metabolites of DINP and DIDP were not analysed in this study. Serious flaws were identified in the study design and statistical analyses employed. Because of these flaws EPA decided not to utilize these studies for hazard and risk assessment.

In a separate study (Main et al, 2006), reported a statistically significant association between levels of DINP metabolites in breast milk of mothers and raised blood levels of leutinizing hormone in infant males. When converting the ratios reported to levels of luteinizing hormone, the levels are actually within normal limits for infants. Other measures of anti-androgenicity, such as reduced testosterone levels were not observed. In fact there was even a trend toward increased testosterone levels which would clearly not support an anti-androgenic effect for DINP. Further, there is still significant scientific uncertainty surrounding the significance of changes in the endpoints examined in the above studies. In addition changes in hormone levels alone are insufficient to conclude on endocrine disrupting properties. Therefore the studies should be considered as scientific research to generate research hypotheses but should not be used for safety evaluation purposes.

6. Definitions for endocrine disrupters

Endocrine disruption has been identified by the European Union (EU) as a criterion to identify substances of equivalent concern under the REACH regulation (Article 57 (f)). Substances of equivalent concern are "substances, such as those having endocrine disruption properties...for which there is scientific evidence of probable serious effects to

humans or the environment which gives rise to an equivalent level of concern to those of the substances listed in points (a) to (e) [Category 2 carcinogens, mutagens, or reproductive agents (CMR), or persistent, bioaccumulative and toxic substances (PBT), or very persistence and very bioaccumulative (vPvB).” The REACH guidance document (“Guidance for the preparation of an Annex XV dossier on the identification of substances of very high concern”) provides a definition of an endocrine disrupter and recommends that given the complexities of the possible mechanisms and effects of endocrine active substances then a weight of evidence approach is needed.

Several definitions of endocrine disruption exist already today. One definition was developed in the late 1990s at an EU sponsored workshop on endocrine disruption in Weybridge, UK. The Weybridge Definition states:

“An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in the endocrine function”.

More recently, the International Programme for Chemical Safety (IPCS) has modified this definition slightly but still with the same overall meaning. The IPCS definition states:

“Endocrine disrupters have been defined as exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism or its progeny secondary to changes in endocrine function.”

This definition is referenced in the EU “Community Strategy for endocrine disrupters” (COM 1999 (706) final).

Further, the IPCS identifies three possible pathways for interference with the endocrine system

- By mimicking the action of a naturally-produced hormone such as oestrogen or testosterone, and thereby triggering similar chemical reactions in the body,
- By blocking the receptors in cells receiving the hormones (hormone receptors), thereby preventing the action of normal hormones; or
- By affecting the synthesis, transport, metabolism and excretion of hormones, thus altering the concentration of natural hormones.

The REACH guidance (“Guidance for the preparation of an Annex XV dossier on the identification of substances of very high concern”) provides a definition of an endocrine disrupter which is almost identical to the above IPCS definition:

“An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.

Based on the three definitions, adverse health effects must be produced as a consequence of endocrine disruption for the conclusion that a substance is an endocrine disrupter. From this it can be concluded that changes in hormone levels by themselves are not sufficient for classification of a chemical as an endocrine disrupter. Hormone levels are changing all the time due to normal cycles and due to external factors e.g. the menstrual cycle in women e.g. consumption of sugar causes insulin levels to rise. Studies which show an increase in hormone levels alone would not be sufficient to classify a substance as an endocrine disrupter, according to the above definitions.

7. Conclusion

- DINP and DIDP are not endocrine disrupters as defined by Weybridge, IPCS and the draft REACH Guidance.

8. References

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Note:

This paper addresses mammalian endocrine disruption. A separate ECPI paper is being prepared which will address endocrine disruption and lack of effects on fish and aquatic organisms.

Information on DINP and the fact it is not an endocrine disrupter is publicly available on the DINP Information Centre website: <http://www.dinp-facts.com/endocrine>

Appendix – The endocrine system

The endocrine system is actually comprised of several discrete systems that are important in regulation of growth, metabolism, development, fluid and mineral balance and reproduction (see table below). Endocrine systems can be comprised of one or several glands that synthesize and secrete substances called hormones into the blood stream. Individual endocrine glands synthesize, store and secrete hormones into the blood streams; some glands secrete more than one hormone, however, these individual hormones are synthesized by discrete cell types within these glands. Once in the blood stream, hormones interact with sites separate from the endocrine gland to produce a desired change in body function.

Brief overview of Major Endocrine Systems			
Body Function	Function	Gland(s)	Hormone(s)
Metabolism	Control glucose levels	Pancreas	Insulin
Growth	Increase size	Pituitary	Growth Hormone
Metabolism	Control metabolic rate	Hypothalamus Pituitary Thyroid	Thyrotropin Releasing hormone Thyroid Stimulating hormone Thyroxine
Mineral Balance	Control of Calcium levels	Parathyroid gland	Parathyroid hormone Calcitonin Vitamin D
Reproduction (female)	Control of menstrual cycle	Hypothalamus Pituitary Ovary (Follicular cells)	Estrogen Progesterone Leutenizing hormone Follicle Stimulating hormone Gonadotropin releasing hormone
Reproduction (male)	Production of sperm	Hypothalamus Pituitary Testes (Leydig cells, Sertoli cells)	Testosterone Leutenizing hormone Follicle Stimulating hormone Inhibin Gonadotropin releasing hormone

All endocrine systems monitor and respond to alterations of the environment within the body. When a stimulus is detected, hormone is released until the stimulus is removed, creating a feedback loop. Some endocrine systems have a simple feedback loop involving one gland and one hormone (i.e., pancreatic release of insulin in response to glucose). For others, the control and release of hormones can be quite complex involving several glands and several hormones (e.g. thyroid hormone control of metabolic rate).

In addition to maintenance of homeostasis, endocrine systems play an important role in normal growth and development. There are critical periods of development in which deficit of hormones results in abnormal development with serious consequences for health. For example absence of thyroid hormone during youth can result in cretinism, resulting in stunted growth and below average intelligence. Additionally, development of the reproductive system and both primary and secondary sexual characteristics requires the synthesis and release of the appropriate hormones at critical times.

Although endocrine systems have been markedly conserved through evolution, there are notable species differences in the operation and maintenance of these systems. These differences are due to the lack of auxiliary structures supporting the endocrine system, or due to differences in how the system as a whole functions. As an example of the former, rats lack thyroid hormone binding globulin, making them more susceptible to perturbations in thyroid hormone levels. For the latter, control of the female reproductive cycle is radically different between rodents and primates. In rodents female rats undergo an oestrous cycle, whereas primates go through a menstrual cycle. The same hormones are used to control different female reproductive cycles.