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Ms. Cynthia Oshita
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RE: Hazard Identification Document (HID) for Diisononyl Phthalate (DINP)

Dear Carcinogen Identification Committee (CIC),

The preface of the Evidence on the Carcinogenicity of Diisononyl Phthalate (DINP) 2013 report clearly states:

> *"Proposition 65 requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. It specifies that "a chemical is known to the state to cause cancer … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer ...."*

Although several hypotheses are presented, the mechanistic arguments proposed for the carcinogenicity and reproductive toxicity of DINP are not supported by the cited *in vitro* data due to limitations in experimental design. More specifically, proposed mechanistic hypotheses based on papers by Harris *et al*., [1], DeKeyeser *et al*., [2], and Bennasroune *et al*., [3] do not provide compelling data that supports conclusions regarding the carcinogenicity and reproductive toxicity of DINP.

First, the use of DINP in *in vitro* studies is inappropriate. *In vivo*, DINP is rapidly hydrolyzed to monoisononyl phthalate (MINP). Furthermore, MINP is a minor metabolite in humans (2% of administered dose) as it is rapidly metabolized to monohydroxy isononyl phthalate (MHINP, 20%), monooxoisononyl phthalate (MOINP, 10%), and monocarboxy isooctyl phthalate (MCIOP, 10%). Consequently, *in vitro* assays should examine the activity of relevant DINP metabolites identified in humans. All three of the above mentioned papers examined the activity of DINP as opposed to the relevant metabolites in humans.

In addition, the concentrations used in these studies exceed the water solubility of DINP [4], and may induce a false positive response due to stabilization of the luciferase mRNA independent of a receptor. Non-receptor mediated signals have been reported for test substances at excessive concentrations due to over activation of the reporter gene [5, 6]. Both the United States Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (EDSP) and the Organization for Economic Cooperation & Development (OECD) recommend avoiding test substance concentrations that are insoluble or induce excessive cytotoxicity (>20%) in transactivation assays [7, 8].

Moreover, the *in vitro* ER-mediated activity of DINP reported by Harris *et al*. [1] was not confirmed in estrogen receptor competitive binding, yeast transactivation, Gal4 chimeric receptor transactivation, and *in vivo* uterotrophic assays [9]. These results are not consistent with the modest estrogenic activity reported by Bennasroune *et al*., [3].

Collectively, results from these in vitro papers are limited and do not provide evidence supporting the proposed mechanisms of carcinogenicity and reproductive toxicity of DINP. Lastly, as recommended by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and adopted by EPA's EDSP, *in vitro* data can only identify the potential of activity of a chemical that warrants further *in vivo* testing.

Consequently, there is insufficient evidence clearly demonstrating through scientifically valid testing according to generally accepted principles that DINP represents a relevant cancer or reproductive toxicant risk to humans.

Sincerely,

Tim Zacharewski

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