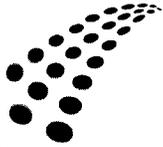


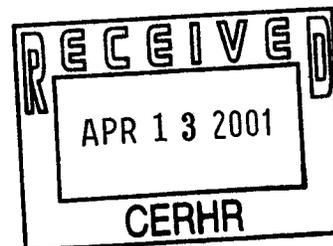
COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

April 11, 2001


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Subject: Supplemental Comments on the CERHR Expert Panel review of DINP

Dear Drs. Shelby and Moore:

In December 2000, the American Chemistry Council Phthalate Esters Panel (PE Panel) provided comments on the evaluations of seven phthalate esters made available by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP CERHR) on its website in October 2000. Among these comments, the PE Panel brought to your attention two publications (Gray et al., 2000; Blount et al., 2000) relating to male reproductive development and exposure to DINP, respectively. As these two issues had been identified by the Expert Panel as critical data needs for DINP, we believed that the papers would be of particular interest to the CERHR. We also expressed the view that, as the data contained within these papers substantially addressed the concerns raised by the Expert Panel, no further testing of DINP was warranted, and that the critical data needs section of that monograph should be modified.

More recently, the groups represented by the Gray and Blount papers have provided additional data which, in our view, further substantiates our request for modifications to the critical data needs section. Accordingly, we have prepared some supplemental comments which, we hope, will be taken into consideration as the NTP CERHR develops its summary report on DINP.

The paper by Blount et al. (2000) reported results of urinary levels of phthalate metabolites, and, in particular found that the levels of DINP metabolites were very low. In two accompanying letters to the editor (David, 2000; Kohn et al., 2000), the urinary metabolite levels were used to estimate external exposures. Both letters estimated that the 95th percentile exposures to DINP would be less than 2 ug/kg/day. This confirmed the CERHR estimate that exposures to DINP would be less than the 3-30 ug/kg/day estimate for DEHP exposure, and demonstrated that the exposures of the general population to DINP are very low. The data published by Blount et al. (2000) have been further substantiated by the CDC in its publication



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of urinary metabolite data from more than 1000 individuals in its National Report on Human Exposure to Environmental Chemicals (CDC, 2001). Although the CDC report did not list a 95th percentile value, the urinary metabolite level at the 90th percentile (4.3 ug/l) is equivalent to an external exposure of 0.6 to 1.0 ug/kg/day for the general population.¹ Thus there is now solid documentation that exposures of the general population to DINP are very low.

Along the same lines, we had previously brought to your attention data on phthalate absorption in humans previously only available in abstract form (Anderson et al., 2000). These data, which demonstrate that absorption of phthalate monoesters by humans is well below that in rodents even at relatively low exposure levels, are now being published and provide additional evidence that internal levels of phthalates in humans are very low (Anderson et al., 2001). For example, Anderson et al. state: "For dioctylphthalate (sum of the 2-ethylhexyl and isooctyl species) the yield was 14 and 12% of the low and high dose excreted as mono-octylphthalate." In contrast, in rodents urinary excretion would be approximately 50% (Rhodes et al., 1986; Astill et al., 1989). Thus, even at exposure levels which are low, approximating those encountered by the general population, the amount of phthalate absorbed by humans is much less than that absorbed by rodents.

The paper by Gray et al. (2000) provided some data relating to the effects of DINP on male reproductive development. Based on this study, conducted at a single dose level of 750 mg/kg/day, Gray et al. reported a significant increase in males with areolas (22% vs. 0% in controls, $p < 0.01$) and also an increase in males with malformations (7.7%, $p < 0.04$). In the latter case, of 52 males examined, 2 had retained nipples, one had small testes and one had testicular atrophy. There were no effects on offspring body weights, anogenital distance, testes weights, preputial separation, serum testosterone levels; no effects on reproductive organ weights; no evidence of undescended testes, prostatic or vesicular agenesis, abnormalities of the gubernacular cord; and no reports of cleft phallus, vaginal pouch, or hypospadias. (Further discussion of this paper, which was included in our previous comments, is attached as an appendix to this letter.)

At the recent Society of Toxicology meeting, Gray's group reported results of studies of the effects of DINP given orally at 1000 and 1500 mg/kg/day (Ostby et al., 2001). Female weight gain during gestation and lactation was reduced by approximately 10% at both treatment levels; offspring body weight was unaffected at 1000 mg/kg/day but reduced by 10% in the 1500 mg/kg/day group. There was a large increase in areolas (55% at 1000 and 70% at 1500 mg/kg/day), but also a relatively high level in the controls (14.7%). There were also small but statistically significant reductions in anogenital distance and age at preputial separation in the 1500 mg/kg/day group, but these parameters were not different from control at 1000 mg/kg/day.

The necropsy results revealed increased nipple retention in both groups, and small but statistically significant reductions in weights of seminal vesicles and levator ani plus

¹ The range reflects the slightly different values provided by the two methodologies reported by David et al. (2000) and Kohn et al. (2000).

Drs. Michael Shelby and John Moore

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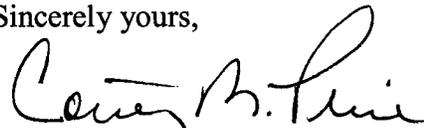
bulbocavernosus muscles in the 1500 mg/kg/day group. Weights of testes, ventral prostate, epididymis and bulbourethral glands were unaffected.

The histological examination revealed a small number of individuals in each group with lesions in the testes or secondary sexual organs, but there was no strong evidence for dose-response. In particular, there was no confirmation that small testes or testicular atrophy were associated with treatment. When these data are compared to the previous publication (Gray et al., 2000), it becomes apparent that baseline values for those parameters under consideration as indicators of anti-androgenic effects and/or male reproductive development need to be established before the toxicological consequences of small changes in such parameters can be confidently interpreted. That is, the incidence in controls in the more recent data indicates that some previous observations in treated animals may have been due to normal variation.

It is our view that the critical data needs for DINP identified by the Expert Panel have now been substantially satisfied, and that section of the CERHR report should be modified. Further, these additional data bear on the conclusions of the Expert Panel that were determined at the meeting in August 2000. The Expert Panel expressed minimal concern for the potential for developmental and reproductive effects in the human population. However, this was tempered in part by the absence of studies of sensitive indicators of male reproductive development and by the "moderate" confidence in the NOAEL for reproductive toxicity. The results now available for Gray's studies are, in fact, quite consistent with the results of the previously published two generation study (Waterman et al., 2000), and should, therefore, resolve some or all of the uncertainty expressed by the Expert Panel. Although Gray has not established a no effect level for areola retention, the low level of effects at 750 mg/kg/day indicate that, if this is not the no effect level, it must be close. Further, these data demonstrate that the effects on male reproductive development were not the most sensitive effects produced by DINP and would have no influence on risk assessments. As the NOAEL for all effects is in the range of 100-200 mg/kg/day, and human exposure is in the range of 1-2 ug/kg/day, the level of concern is better described as "negligible" than "minimal."

Please let us know if we can provide additional information. You may call Marian K. Stanley, Manager of the Phthalate Esters Panel, at (703) 741-5623 or e-mail her at Marian_Stanley@americanchemistry.com.

Sincerely yours,



Courtney M. Price
Vice-President, CHEMSTAR

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Ostby, J. et al. (2001). Investigation of the ability of diisononyl phthalate (DINP) to alter androgen-dependent tissue development in Sprague-Dawley rats. The Toxicologist 60:225.

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Appendix
Extract from The Phthalates Esters Panel December 11, 2000
Comments to NTP CERHR, Concerning the Gray Study

General Comment

During the DINP discussions the Expert Panel considered that data on male reproductive development were insufficient. Although the published information provided no evidence of such effects, the Panel took note of an abstract which reported an increased incidence in rats of malformations of the male reproductive system. In the absence of published data, the Expert Panel expressed only moderate confidence in the NOAEL for reproductive toxicity and expressed the desire that such studies be conducted along with a better assessment of human exposure. Recently a paper has been published (Gray *et al.*, 2000)¹ which did assess developmental indicators at 750 mg/kg/day. There was a statistically significant increase in areolas at PND 13, and, according to the authors, a small increase in malformations. None of the other parameters measured in the study were affected by treatment. The availability of these data should increase the confidence of the Expert Panel in the selection of NOAELs and should also obviate the need for any further tests of this type. Further, urinary metabolite studies indicate that human exposures are many orders of magnitude below the effect levels in rodent studies (Blount *et al.*, 2000; David, 2000; Kohn *et al.*, 2000).² Accordingly, the Phthalate Esters Panel believes that current production and use of DINP pose no risks to human reproduction or development.

...

Comments Based on Recently Published Data

The CERHR Expert Panel Review of DINP referred to data from Gray's laboratory, available only in abstract form during the deliberations (Ostby *et al.*, 2000).³ Although the conclusions from the abstract were cited in several places (*e.g.*, last paragraphs of sections 3.2 and 4.2) as evidence that DINP has an effect on male reproductive development, the absence of such data in the published literature concerned the Expert Panel, diminishing their confidence in their overall confidence in NOAELs, and resulting in a recommendation for additional studies listed in the critical data needs section. As the data from Gray's laboratory have now been

¹ Gray, L. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP and DINP but not DEP, DMP or DOTP alters sexual differentiation of the male rat. *Toxicological Sciences* 58:350-365.

² Blount, B., *et al.* (2000). Levels of seven urinary phthalate metabolites in a human reference population. *Environmental Health Perspectives* 108:979-982; Kohn, M., *et al.* (2000). Human exposure estimates for phthalates. *Environmental Health Perspectives* 108:A440-A442 (correspondence); David, R. (2000). Exposure to phthalate esters. *Environmental Health Perspectives* 108:A440 (correspondence).

³ Ostby, J. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP, DINP but not DEP, DMP or DOTP permanently alters androgen-dependent tissue development in Sprague-Dawley rats. Triangle Consortium on Reproductive Biology, January 29, 2000.

published (Gray *et al.*, 2000),⁴ the Expert Panel should fully evaluate those data and incorporate them in the monograph as suggested below.

As reported by Gray, female Sprague-Dawley (SD) rats were given DINP (CAS # listed as 68515-48-0) by oral gavage from GD14 to PND 3 at a single treatment level, 750 mg/kg/day. The offspring were examined at various times until terminal sacrifice at times ranging from 3-7 months of age. The parameters which were examined included:

- (a) Body weight and anogenital distance on PND 2 – These parameters were unaffected by DINP treatment.
- (b) Testicular examination on PND 3 – Testes weights of DINP-treated male offspring were similar to control.
- (c) Inguinal examination of male pups – It was reported that one DINP-treated male offspring had “suspected” “hemorrhagic testes”, but this was not confirmed by histologic examination.
- (d) Examination for areolas on day 13 – The incidence of areolas (22%) was reported as significantly different from control at $p < 0.01$.
- (e) Examination of onset of puberty (preputial separation) – Not affected by treatment.
- (f) Determination of serum testosterone levels at terminal sacrifice – Not affected by treatment.
- (g) Examination for retained nipples, cleft phallus, vaginal pouch and hypospadias – Of 52 male offspring examined, 2 had retained nipples; none had cleft phallus, vaginal pouch or hypospadias.
- (h) Internal examination for undescended testes, atrophic testes, epididymal agenesis, prostatic and vesicular agenesis, and abnormalities of the gubernacular cord – One of the male offspring was reported to have had bilateral testicular atrophy and another exhibited epididymal agenesis with hypospermia and fluid filled testes. None of the 52 male offspring examined had undescended testes, prostatic and vesicular agenesis or abnormalities of the gubernacular cord.
- (i) Body weights and weights of organs including ventral prostate, levator ani plus bulbocavernosus muscles, seminal vesicles, and epididymides – Weights of all organs, including all of the reproductive organs were similar to controls.
- (j) Sperm counts – It was not clear from the report whether or not sperm counts of DINP-treated animals were examined. The paper was silent on the results of sperm analysis for all substances except for BBP and DEHP for which sperm counts were reported to be reduced, but the data were not provided.

⁴ Gray, L. *et al.* (2000). Perinatal exposure to the phthalates DEHP, BBP and DINP but not DEP, DMP or DOTP alters sexual differentiation of the male rat. *Toxicological Sciences* 58:350-365.

The abstract which was cited by the CERHR (Ostby *et al.*, 2000) contains a statement that “males in the ... DINP (7.7%, $p < 0.04$) treatment group displayed malformations of the testis, epididymis, accessory reproductive organs and external genitalia.” As now reported in the full publication, 4 (of 52) treated male offspring were considered by the authors to have been malformed. These included 2 with retained nipples, one with “small” testes, and one with testicular atrophy. The statistical analysis compared the total incidence of offspring considered malformed against the controls rather than making comparisons for each anomaly. The statistical evaluation indicated $p < 0.05$ when the data were compared on an individual basis and $p < 0.06$ for a litter-based comparison. No data on historical control incidences were provided. Given the low incidence of anomalies, it is difficult to determine whether these are spontaneous or treatment related. Further, the validity of pooling all affected individuals for statistical analysis seems questionable. Certainly, the effects evaluated individually would not be significantly different from control. We believe that these results are marginal and do not form a basis for strong conclusions of the effect of DINP on male reproductive development.

More important is the question of whether this publication provides any information on reproductive toxicity beyond that provided by the two generation reproduction study previously reported by Waterman *et al.* (2000). Gray’s study utilized oral gavage in contrast to dietary administration in Waterman and at a somewhat higher dose level (in Waterman the estimated maternal dose on GD 14-21 was 543 mg/kg and that on PND 0-4 was 672 as compared to 750 mg/kg in Gray). Nevertheless, Gray confirmed one of the most important findings of Waterman, *i.e.*, that DINP treatment during the period of male reproductive development has no effect on male reproductive organs. More specifically, Gray found no effects on weights of testes or accessory reproductive organs, and identified only 2 rats (of 52) with what he considered to be malformed testes. Waterman also found weights of testes and accessory organs to be unaffected. In addition, Waterman found that within the parental generation, one male, from the control group, had unilateral focal testicular atrophy. In the F1 generation there were two males with diffuse unilateral atrophy and testicular degeneration; one from the control group and one from the high dose group. As similar effects were found at the same incidence in the treated and control groups, these findings were judged by Waterman to be incidental.

The one clear difference between these two studies is that Gray found an increase in areolas in 13-day old male pups. However, the toxicological significance of this effect is questionable since it appeared to be substantially reversible. Among the 13 day old male offspring, 22% had areolas; at terminal sacrifice, 2 (of 52) or 4% of the males had retained nipples. Although the frequency of areolas was increased, the demonstration that DINP had no effects on fertility, and minimal effects on male reproductive development should provide the Expert Panel with the information that these minor effects have no bearing on human reproductive risk. That males with areolas can reproduce was shown by Schilling (1999)⁵ in a study of the potential reproductive effects of DEHP.

The above having been said, these data seem more relevant to the overall assessment of developmental toxicity than reproduction. There was a significant increase in frequency of areolas at 750 mg/kg, but this appeared to have been substantially reversed by terminal sacrifice.

⁵ Schilling, K. *et al.* (1999). Reproduction toxicity of di-2-ethylhexyl phthalate. *The Toxicologist* 48:147-148.

Although no NOAEL was defined, the level associated with this effect was higher than other developmental effects considered by the Expert Panel, and, therefore, should not influence the overall evaluation of developmental toxicity. The reproductive NOAEL had previously been defined by the absence of effects on fertility and/or reproductive organs as reported by Waterman. Gray provided no new data on fertility and confirmed the absence of effects on reproductive organ weights. Although Gray reported a low incidence of testicular effects, the marginal nature of those findings along with the absence of effects in Waterman indicate that these data should not be used for NOAEL determination. That, in effect, would leave in place the existing LOAELs and NOAELs, but should increase the Expert Panel confidence. With more confidence in both the toxicity and exposure information, it would be more appropriate to change the concern level to negligible.