Report to the

U.S. Consumer Product Safety Commission

by the

CHRONIC HAZARD ADVISORY PANEL ON DIISONONYL PHTHALATE (DINP)

June 2001

U.S. Consumer Product Safety Commission

Directorate for Health Sciences

Bethesda, MD 20814

Lawrence Livermore National Laboratory

HEALTH & ECOLOGICAL
ASSESSMENT DIVISION

May 24, 2001

Hon. Ann Brown, Chairman
U.S. Consumer Product Safety Commission
4330 East West Highway
Bethesda, MD 20814

Dear Chairman Brown:

On behalf of the Chronic Hazard Advisory Panel on Diisononyl Phthalate (DINP), I am pleased to transmit the Panel's report.

The Panel concluded that DINP is clearly carcinogenic to the rodent, inducing hepatocellular carcinoma in rats and mice of both sexes and mononuclear cell leukemia in male and female rats with limited evidence of carcinogenicity based on renal tubular carcinoma in male rats. DINP, however, is not genotoxic and causes liver cancer in rodents through a peroxisome proliferator-activated receptor (PPARα). The PPARα mechanism is pronounced in rodents, but believed not readily induced in humans under current exposure conditions involving consumer products. The human risk is therefore seen as negligible. DINP induces renal tubular carcinoma by an α2μ–globulin mechanism that is a rodent specific mechanism and unlikely to be relvant to a determination of human risk. Mononuclear cell leukemia also may be a rodent-specific cancer of unclear relevance to a determination of human risk. The CHAP concludes that humans do not currently receive DINP doses from DINP-containing consumer products that are plausibly associated with a significant increase in cancer risk.

Although DINP studies in rats indicate that it is a teratogen and reproductive toxicant, the risk to reproductive and developmental processes in humans due to DINP exposure is extremely low or non-existent.

The critical endpoint to use to determine an ADI is spongiosis hepatis in male F344 rats. The ADI based on a 5th-percentile Benchmark Dose ($BD_{0.05}$) and a 100-fold conbined uncertainty/adjustment factor would be 0.012 mg kg^{-1} d^{-1}. There may be a DINP risk to young children who routinely mouth DINP-plasticized toys for 75 minutes per day or more. For most children, exposure to DINP from DINP-containing toys would be expected to pose a minimal to non-existent risk of injury.

In its report, the Panel has summarized the information available from published reports and scientists working on this subject. The Panel has addressed questions

posed by the Commission. Finally, the CHAP has indicated areas of uncertainty associated with both the hazard and exposure. Additional data in these areas would permit a more definitive risk assessment of DINP.

The report represents the views of a majority of the CHAP, but each member of the CHAP does not necessarily agree with all points presented in the report.

Sincerely,

Kenneth T. Bogen, Chairman
Chronic Hazard Advisory Panel on DINP

Approved:

Kenneth Bogen, Chairman

Kim Boekelheide, Vice Chairman

Michael Cunningham

Benjamin Jackson

Jeffrey Peters

Janardan Reddy

Lauren Zeise

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Chronic Hazard Advisory Panel on Diisononyl Phthalate

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I. EXECUTIVE SUMMARY

Diisononyl Phthalate (DINP) is a complex of branched C-9 isomers that is used as a general purpose plasticizer to render polyvinyl chloride (PVC) flexible. It has a broad range of applications in toy manufacturing, construction, and general consumer product markets.

The Consumer Product Safety Commission convened a panel of scientific experts to determine whether DINP in consumer products poses a chronic hazard and, if feasible, indicate the probable harm to human health resulting from exposures to DINP. This is the final report of that panel, the Chronic Hazard Advisory Panel (CHAP) on Diisononyl Phthalate (DINP). On any particular issue, a range of viewpoints was held among panel members. This document reports the majority view for each issue, which typically was not unanimous.

Human exposure to DINP may occur via oral, dermal, and inhalation exposure routes. Based upon the physiochemical characteristics of DINP and limited monitoring data, general environmental exposure to DINP in the U.S. adult population is likely to be substantially lower than exposure to DEHP, which is estimated at 0.003-0.03 mg $kg^{-1}d^{-1}$ (milligrams per kilogram body weight per day). The most significant exposures to DINP are likely to occur from the use of consumer items that consist of flexible plastic plasticized using DINP. These consumer items currently include PVC toys routinely mouthed by young children. Mouthing of DINP-containing toys may result in ingestion exposures of 0.07 and 0.28 mg $kg^{-1}d^{-1}$ in reasonably highly exposed subsets of children 19-36 months old and 0-18 months old, respectively. Dermal uptake of DINP may also occur through prolonged contact of DINP containing products with skin or mouth. However, detailed data on the prevalence of DINP in consumer products that are in sustained contact with skin, such as sandals and rainwear, are not available, and there is fundamental uncertainty concerning the magnitude of dermal DINP uptake. Therefore, estimation of potential dermal exposure from such products remains speculative.

DINP belongs to a class of structurally diverse chemicals called peroxisome proliferators. These chemicals interact with a cellular receptor involved in lipid metabolism (i.e., peroxisome proliferator-activated receptor-α) to induce the proliferation of peroxisomes in addition to other cellular responses. Because rodents and humans differ in responses resulting from the activation of this receptor, a critical issue for the evaluation of rodent toxicity studies to predict human risk is whether the receptor is involved. The non-cancer toxicities discussed below are not believed to involve activation of this receptor.

Of the systemic effects from chronic exposure to DINP, spongiosis hepatis, a degenerative lesion of the liver, is the most sensitive endpoint. The no observed adverse effect levels (NOAELs) identified in laboratory animals exposed to DINP were 15 mg $kg^{-1}d^{-1}$ in one study and 88 mg $kg^{-1}d^{-1}$ in a second study.

No human data were located on the reproductive or developmental toxicity associated with DINP exposure; therefore, the evaluation of these endpoints has relied upon animal studies**.** Using standard assays of prenatal oral exposure of rats to DINP, developmental toxicity consisting of renal and skeletal abnormalities occurred with NOAELs of 100 and 200 mg $kg^{-1}d^{-1}$ in the two standard prenatal developmental studies in rats. A two-generation study in the rat suggested an adverse effect upon pup weight gain with a lowest observed adverse effect level (LOAEL) of 250 mg $kg^{-1}d^{-1}$. In a recently published report of high dose exposure of rat dams to DINP during critical stages of fetal male reproductive tract development, male reproductive tract malformations consistent with an antiandrogenic effect were observed. Because of the large margin between doses to pregnant women and those expected to be without effect in the animal assays, the risk to reproductive and developmental processes in humans due to DINP exposure is extremely low or non-existent.

Collectively, the majority of data indicate that DINP is non-genotoxic, consistent with results obtained for other peroxisome proliferators. DINP has been tested in bacterial mutation assays and mammalian gene mutation assays *in vitro*, with or without metabolic activation, and found to be non-mutagenic. DINP has also been evaluated in both *in vivo* and *in vitro* cytogenetic assays with results supporting the idea that DINP is not genotoxic. Lastly, *in vitro* analysis of unscheduled DNA synthesis in rat hepatocytes which are known target cells of peroxisome proliferators provided no evidence of mutagenicity caused by DINP. Still, the peroxisome proliferation that results in rodents from receptor activation following DINP exposure may cause gene damage by increasing the level of hydrogen peroxide in the cell.

DINP is clearly carcinogenic to the rodent, inducing hepatocellular carcinoma in rats and mice of both sexes, renal tubular carcinoma in male rats, and mononuclear cell leukemia in male and female rats. Because nearly all male Fischer rats develop studies testicular interstitial cell tumors, the technical grade DINP studies in Fischer rats provide no information on the potential for development of these tumors. The chemical has not been tested for carcinogenicity in young rodents, an important limitation given that infants and toddlers are the ones most exposed to DINP. Chronic carcinogenicity studies have not been conducted in non-rodent species. Because of the lack of confidence in the relevance of the DINP rodent studies to humans, studies in species believed to produce results of greater relevance are clearly needed.

Peroxisome proliferators are a structurally diverse group of non-mutagenic chemicals that induce predictable pleiotropic responses including the development of liver tumors in rats and mice. These nonmutagenic chemicals interact variably with peroxisome proliferator-activated receptors (PPARs), which are members of the nuclear receptor superfamily. Evidence derived from PPARα gene disruption indicates that of the three PPAR isotypes (α, β/δ, and γ), the isoform PPARα is essential for the pleiotropic responses induced by peroxisome proliferators including the development of hepatocellular carcinomas. While the evidence is overwhelming that events downstream of PPARα activation lead to liver cancer in rodents, the relative roles of the possible, nonexclusive, downstream mechanisms – oxidative stress, apoptosis, and cell proliferation, with or without Kupffer

cell involvement – are unclear. DINP is classifiable as a hepatic peroxisome proliferator and in that regard the liver tumors developing in rats and mice chronically exposed to DINP can be mechanistically related to PPARα activation. The PPARα-mediated mechanism of hepatocarcinogenesis is pronounced in rodents, but believed not readily induced in humans, especially at the doses resulting from current use of consumer products. The human risk was therefore seen as negligible or non-existent. The male rat α2μ-globulin mechanism of action for the production of rat kidney tumors has been postulated. Criteria for supporting an α2μ-globulin mechanism of action were applied and found to be met. The renal tumors in male rats at the high dose of DINP were therefore treated as rat specific and were not used to predict human risk. The mononuclear cell leukemia (MCL) in Fischer 344 (F344) rats was viewed of questionable significance and was not used in human risk prediction.

The available data indicate that humans do not receive DINP doses from current uses of DINP-containing consumer products that are associated with a significant increase in cancer risk. The most sensitive toxicity endpoint is spongiosis hepatis, observed in male F344 rats. A Benchmark Dose (BD_{05}) estimate of 12 mg $kg^{-1}d^{-1}$ has been calculated. The corresponding acceptable daily intake (ADI) would be 0.120 mg $kg^{-1}d^{-1}$ based upon the application of a 100-fold combined uncertainty/adjustment factor. Background exposures to DINP and other phthalates could not be considered due to scientific uncertainties (see Section XI). One of the two estimates of plausible upper-bound DINP exposure is greater than the recommended ADI of 0.12 mg $kg^{-1}d^{-1}$. Namely, the estimate of 0.28 mg $kg^{-1}d^{-1}$ for ingested DINP among any children 0-18 months old who mouth PVC plastic toys containing DINP for 3 hours/day exceeds the recommended ADI. This implies that there may be a DINP risk for any young children who routinely mouth DINP-plasticized toys for 75 minutes/day or more. For the majority of children, the exposure to DINP from DINP containing toys would be expected to pose a minimal to non-existent risk of injury.

The exposure estimates addressed oral exposures only. Dermal exposure is expected from products plasticized with DINP in prolonged contact with external skin or oral mucosa; however the magnitude of this exposure is uncertain. The CHAP recommends experiments be undertaken to reduce this important source of uncertainty in the risk characterization.

The CHAP is conveying these findings in the series of questions and answers provided below. As noted above, the answers to the questions represent a majority view of the CHAP and are not necessarily the view of every member of the CHAP.

1. What is the critical endpoint to use to determine the ADI?

    The critical endpoint is spongiosis hepatis in male F344 rats.

2. What is the Acceptable Daily Intake (ADI) for DINP?

The ADI based on a BD_{05} and a 100-fold combined uncertainty/adjustment factor would be 0.120 mg $kg^{-1}d^{-1}$.

3. Are the results of the carcinogenicity bioassays on DINP adequate and sufficient to conclude that DINP is a rodent carcinogen?

Yes, DINP is clearly carcinogenic to the rodent, inducing hepatocellular carcinoma in rats and mice of both sexes and mononuclear cell leukemia in male and female rats. There is limited evidence of carcinogenicity based upon renal tubular carcinoma in male rats.

4. Is the carcinogenicity of DINP in rodents relevant to a determination of carcinogenicity in humans?

The hepatocarcinogenicity of DINP in rodents may be relevant to a determination of carcinogenicity in humans. Renal tubular carcinoma does not appear to be relevant to a determination of carcinogenicity of DINP in humans. Mononuclear cell leukemia is of unclear relevance for a determination of carcinogenicity of DINP in humans. See #6 for a further explanation.

5. Is DINP genotoxic?

The majority of data indicate that DINP is non-genotoxic, consistent with results obtained from analysis of other chemicals which function similarly to cause liver cancer in rodents through peroxisome proliferator-activated receptor-α (PPARα). The peroxisome proliferation that results in rodents from receptor activation following DINP exposure may cause gene damage by increasing the level of hydrogen peroxide in the cell.

6. What is the mechanism by which DINP causes cancer in rodents and what is the relevance of such data to a determination of human risk?

DINP appears to induce liver cancer in rodents by a PPARα-mediated mechanism that is pronounced in rodents, but believed not readily induced in humans under current exposure conditions involving consumer products. The human risk was therefore seen as negligible.

DINP appears to act by an α2μ–globulin mechanism to cause renal tubular carcinoma. The CHAP considers this to be a rodent specific mechanism and unlikely to be relevant to a determination of human risk. Mononuclear cell leukemia also may be a rodent-specific cancer of unclear relevance to a determination of human risk.

7. What is the carcinogenic risk to humans from exposure to DINP in consumer products?

> The CHAP concludes that humans do not currently receive DINP doses from DINP-containing consumer products that are plausibly associated with a significant increase in cancer risk.

8. Is DINP a developmental or reproductive toxicant and would the exposures from consumer products result in developmental or reproductive risks?

> Studies in rats at a high dose indicate an adverse effect on pup weight gain and male reproductive tract malformations consistent with an antiandrogenic effect. However, because of the large margin between doses to pregnant women and those expected to be without effect in the animal assays, the risk to reproductive and developmental processes in humans due to DINP exposure is extremely low or non-existent.

9. Is there evidence that children are more sensitive to the effects of DINP and if so how should that be incorporated into any risk determination?

> No data are available on the effect of DINP on children or immature experimental animals, nor are there data that indicate that immature animals are more sensitive to causes of spongiosis hepatis, the critical endpoint used by the Panel in the DINP risk assessment.

10. How should background levels of DINP and other phthalates be incorporated into a determination of risk?

> There are no data on the interaction or additivity of dialkyl phthalate-induced toxic effects. Even if they act through a common mechanism, DAP effects are not necessarily additive, although the assumption of additivity for low exposure levels is a generally accepted conservative approach to addressing this source of uncertainty, as well as one that has theoretical support in the case that damage occurs by statistically independent increments.
>
> However, because of the difficulty in developing reliable estimates of phthalate exposure for the population of interest (infants and toddlers) and uncertainties on how exposure estimates should be combined for comparison with the ADI, further explicit consideration of environmental background DAP exposures is not undertaken.

11. What conclusions, if any, can be reached about the skin penetration of DINP as a result of dermal contact? Should potential risks from dermal exposures be evaluated in the same manner as those from oral exposure?

Dermal uptake of DINP may occur through prolonged contact of DINP containing products with skin or mouth. However, detailed data on the prevalence of DINP in consumer products that are in sustained contact with skin, such as sandals and rainwear, are not available, and there is fundamental uncertainty concerning the magnitude of dermal DINP uptake. Therefore, estimation of potential dermal exposure from such products remains speculative.

12. Is the available exposure information adequate to permit the Panel to estimate the probable harm, if any, to human health that will result from exposure to DINP from the "reasonable and foreseeable" use of consumer products?

Estimated DINP exposures to children through toys and/or bedding/shoes/ clothing, and to adults from shoes/clothing, are preliminary at best. Recognizing the limitations of the data, nevertheless, a prediction about the potential oral exposure to children under the age of three to certain consumer products can be made. Exposure information is inadequate to make predictions about dermal exposure.

13. If such an estimate were made, what methodologies were used in estimating the magnitude of the risk and what was the rationale for adopting that methodology?

A safety factor approach was applied to a non-cancer endpoint. To induce liver cancer, DINP acts by a PPARα mechanism that is pronounced in rodents and that is not readily induced in humans under current exposure conditions. Thus, the human risk from cancer was seen as insignificant.

14. What are the uncertainties attendant with determining the risk to children from exposure to DINP in consumer products?

There are uncertainties associated both with the determination of exposure and the determination of hazard. Those associated with exposure include:

- lack of knowledge about what portion of toys contain DINP
- lack of knowledge about what other consumer products contain DINP
- lack of knowledge about how much DINP migrates out of toys and other consumer products
- uncertainties about how much time each day a child spends with toys and other DINP containing objects in their mouths
- lack of knowledge about how much, if any, DINP would be dermally absorbed

The uncertainties associated with the hazard include:

- the degree to which spongiosis hepatis in rodents is relevant to humans
- how to extrapolate an effect from a lifetime exposure in rodents to a two-to-three year exposure in young children
- lack of knowledge of effects of early in life exposures; there are no toxicological data for exposures corresponding to infancy and toddler years
- lack of knowledge of effects in non-rodents; there are no chronic studies in non-rodent mammals
- lack of knowledge of PPARα expression and related responses in the young; there are no data in human infants and children and scant data in non-human species
- lack of knowledge on mechanisms by which PPARα induces rodent liver tumors

15. What is the risk to children from the oral exposure to DINP?

One of the two estimates of plausible upper-bound DINP exposure listed in Table IV-7 (Section IV) is greater than the ADI of 0.12 mg $kg^{-1}d^{-1}$ recommended above for DINP. Namely, the estimate of 0.28 mg $kg^{-1}d^{-1}$ for ingested DINP among any children 0-18 months old who mouth PVC plastic toys containing DINP for 3 hours/day exceeds the recommended ADI. This implies that there may be a risk of health effects from DINP exposure for any young children who routinely mouth DINP-plasticized toys for 75 minutes/day or more. For the majority of children, the exposure to DINP from DINP containing toys would be expected to pose a minimal to non-existent risk of injury. Further research addressing topics listed above (see question #14) could reduce the uncertainty associated with this characterization of DINP risk from consumer products.

II. INTRODUCTION

The Commission voted on December 17, 1998 to convene a Chronic Hazard Advisory Panel (CHAP) on Diisononyl Phthalate (DINP). A CHAP is a panel of scientific experts that reviews scientific data and other relevant information regarding any potential risks of cancer, birth defects, or gene mutations from the presence of a chemical (in this case DINP) in consumer products. The mission of this panel is to determine whether DINP is a carcinogen, mutagen, or teratogen or poses some other chronic hazard and, if feasible, estimate the probable harm to human health that will result from exposure to DINP. Activities of the CHAP were conducted in accordance with sections 28 and 31 of the Consumer Product Safety Act (CPSA)[*], 15 U.S.C. 2077, 2080.

Candidate members for the CHAP were selected by the President of the National Academy of Sciences. From the thirty-three nominees, the U.S. Consumer Product Safety Commission selected seven Panel members. Immediately subsequent to their selection, one of the panel members indicated he was no longer able to participate. On March 8, 2000, the Commission selected a replacement who, just prior to the first meeting, was told by his employer he could not participate. The Commission selected a replacement for him on May 25, 2000. The seven panel members chose Dr. Kenneth Bogen as the Chairman and Dr. Kim Boekelheide as the Vice Chairman. The CPSA requires that Panel members be scientists who have demonstrated the ability to critically assess chronic hazards and risks to human health presented by the exposure of humans to toxic substances or by the exposure of animals to such substances. Members may not be officers or employees of the United States (other than employees of the National Institutes of Health, the National Toxicology Program (NTP), or the National Center for Toxicological Research) or receive compensation from or have any substantial financial interest in any manufacturer, distributor, or retailer of a consumer product.

In December 1998, the Commission staff completed an analysis, "The Risk of Chronic Toxicity Associated with Exposure to Diisononyl Phthalate (DINP) in Children's Products." As a result of this analysis and recommendations made by CPSC staff, toy manufacturers voluntarily agreed to remove DINP from rattles and teethers and another phthalate from pacifiers and baby bottle nipples. In addition, a number of large retail chains agreed not to sell rattles, teethers, pacifiers, or baby bottle nipples that contained phthalates. Staff indicated at that time that there were a number of uncertainties in the staff's analysis and recommended that the Commission:

- continue work to develop a laboratory test method that more accurately estimates the amount of phthalate released when products are mouthed by children
- conduct additional testing of products intended for children under 3 years of age that contain DINP

[*] Consumer Product Safety Amendments of 1981, Public Law 97-35, Title 12, Subtitle A, 95 Stat. 703, August 13, 1981.

- conduct a more extensive exposure study of the amount of time children mouth products that may contain phthalates
- convene a Chronic Hazard Advisory Panel (CHAP) of independent scientists to study issues related to the chronic toxicity and risk, including the risk of cancer, associated with exposure to DINP in children's PVC products

Additionally, the Commission received a petition (HP 99-1) from the National Environmental Trust and eleven other organizations asking the Commission to ban PVC in children's products. One of the reasons the petitioners gave for the request to ban PVC in children's products was that DINP was used in PVC as a softener and it posed a hazard to children.

On April 26, 2000 the Commission published a Federal Register Notice (FR 65(81): 24458) announcing the first meeting of the CHAP. On May 30, 2000 the Commission published another Federal Register Notice (FR 65(104): 34446) inviting public comment at the next CHAP meeting and requesting information in a number of areas and laying out a series of questions:

1. What is the appropriate Acceptable Daily Intake (ADI) for DINP?
2. Which critical endpoint should be used to determine the ADI?
3. What is the mechanism by which DINP causes cancer in rodents and what is the relevance of these induced neoplasms to human risk?
4. What is the most appropriate measure of the biologically effective dose for DINP-induced liver cancer?
5. What is the appropriate risk assessment model or models to determine human risk? Is there convincing evidence that a linear extrapolation approach for risk assessment is not appropriate for DINP?
6. Is there a differential sensitivity/susceptibility of young children to the effects of DINP? If there is, how should it be incorporated into an assessment of risk?
7. Are there age dependent pharmacokinetic differences?
8. Are DINP rodent cancer bioassay data inapplicable to human hazard identification?
9. Information was requested on the following:
   a. Percutaneous absorption of DINP
   b. Pharmacokinetics of DINP including salivary metabolism
   c. DINP metabolites
   d. Spongiosis hepatis
   e. Total exposure to phthalates in adults and humans
   f. Toxicological interactions between phthalate esters

The CHAP met three times in open session: May 10-11, 2000; June 20-22, 2000; and September 12-13, 2000. At the first CHAP meeting, the Commission staff made presentations on the toxicity of DINP, the completed studies on children's mouthing behavior, the study that the Commission was beginning on children's mouthing behavior, the migration of DINP from toys, and other national and international activities on DINP. The CHAP then discussed the format of the report and what further information it

wanted. Prior to the June meeting, a Federal Register notice was published listing information the CHAP wanted and soliciting comment.

At the June CHAP meeting, presentations were made by Rick Hind, Legislative Director, Toxics Campaign, Greenpeace; Tom Natan, Research Director, National Environmental Trust; Rachel Weintraub, Consumer Advocate, U.S. Public Interest Research Group; Raymond M. David, Ph.D., Chairman, Phthalates Ester Panel, Toxicology Research Task Group; Jerry F. Hardisty, D.V.M., President and Pathologist, Experimental Pathology Laboratories, Inc.; Ruth A. Roberts, Ph.D., Toxicologist, Zeneca Central Toxicology Laboratory; Chris Corton, Toxicologist, Chemical Industry Institute of Toxicology; Rainer Bahnemann, D.V.M., Pathologist, BASF Corporation and member, Phthalates Ester Panel Toxicology Research Task Group; James Klaunig, Ph.D., Professor of Pharmacology and Toxicology, Indiana University School of Medicine; Gary M. Williams, M.D., Professor, Department of Pathology, New York Medical College; and Richard H. McKee, Ph.D., Toxicologist, ExxonMobil Biomedical Sciences, Inc. and member Phthalates Ester Panel Toxicology Research Task Group. The CHAP spent the remainder of this meeting and the September meeting addressing specific issues, drafting and reviewing parts of the report.

III. GENERAL CHEMICAL AND BIOPHYSICAL PARAMETERS

A. Chemistry

DINP is a class of dialkyl phthalate esters (DAPs) that represents a complex of branched, predominantly C-9 isomers. DINP is a complex substance and is assigned different CAS numbers based on the method of manufacture. CAS number 68515-48-0 (designated as DINP-1 in this document) is manufactured from octene that is converted to alcohol moieties consisting mainly of 3,4-, 4,6-, 3,6-, 3,5-, 4,5-, and 5,6-dimethyl-heptanol-1. CAS number 28553-12-0 (DINP-2) is produced from n-butene that is converted primarily to methyloctanols and dimethylheptanols. CAS number 28553-12-0 also represents DINP-3, which is produced from n-butene and isobutene that are converted to alcohols, with 60% consisting of methylethyl hexanols. The Chemical Manufacturers Association (CMA 1999) has stated that although DINP is a complex substance, it is not variable due to the stability of the alcohol manufacturing process. The first two types of DINP mentioned above are considered commercially interchangeable.

DINP is an oily viscous liquid at standard temperature and pressure. Some physical and chemical properties of DINP are shown in Table III-1 (from Staples et al., 1997).

Table III-1. Physical and Chemical Properties of DINP

Property	Value
Chemical Formula	$C_{26}H_{42}O_4$
Molecular Weight	418.62
Melting Point	-48 ^{o}C
Boiling Point	370 ^{o}C
Specific Gravity	0.97
Solubility in Water	Insoluble (0.2 mg/L)
Vapor Pressure	5.4 x 10 $^{-}$7 torr
Log K_{ow}	~9

B. References

Chemical Manufacturers Association (CMA, 1999). CMA Comments of the Chemical Manufacturers Association phthalate esters panel in response to request for public input on seven phthalate esters (1999) FR Doc. 99-9484. Washington, DC: Chemical Manufacturers Association (currently the American Chemistry Council).

Staples, C.A., Peterson, D.R., Parkerton, T.F. and Adams, W.J. (1997) The environmental fate of phthalate esters: A literature review. *Chemosphere* 35:667-749.

IV. CONSUMER EXPOSURE

A. Background Exposure to DINP and Related Compounds

Within the class of dialkyl phthalate esters (DAPs), DINP represents a complex of branched, predominantly C-9 isomers used as a general-purpose plasticizer that renders polyvinyl chloride (PVC) flexible. DINP, therefore, has a broad range of applications in toy manufacturing, construction, and general consumer product markets. DINP is among the primary phthalate esters manufactured worldwide for industrial applications. Di(2-ethylhexyl) phthalate (DEHP), also known as dioctyl phthalate (DOP), and the functionally equivalent plasticizer diisooctyl phthalate (DIOP) comprise roughly half of all DAP manufactured, the remainder including primarily DINP and diisodecyl phthalate (DIDP), which compete with DEHP as commodity general purpose plasticizers, and specialty phthalates such as dibutyl phthalate (DBT) and diisobutyl phthalate (DIBP) (ECPI, 2001). DINP has limited use in food packaging and is not currently used in medical products. Products containing DINP and quantities of DINP used in their production are as indicated in Table IV-1 below.

Human exposure to DINP may occur via oral, dermal, and inhalation exposure routes. Potential exposure to DINP from sources other than consumer products is generally considered negligible, because monitoring data for DINP in air, drinking water, surface and ground waters, food and infant formula, and occupational environments have been at or below the limit of detection (typically 0.01 mg kg^{-1}) (IUCLID, 1998; MAFF, 1998; NTP/CERHR, 2000). Because DINP accounts for ~10-15% of total DAP production (CMA, 1998), it is not clear why DINP is rarely detected in environmental samples and in food. To the extent that production and use of DINP may be increasing relative to other DAPs due to substitution for alternative DAPs such as DEHP, environmental and biodosimetric detection of DINP would be expected to increase. In urine recently sampled from a reference population of 289 U.S. adults, isononyl phthalate monoester (a metabolite of DINP) was detected in samples collected from more than 5% of the tested population. The geometric mean and maximum urinary concentrations were 1.5 and approximately 80 ppb, respectively, for the entire population tested (Blount et al., 2000).

The greatest exposures to DINP in consumer products may result from products designed for children. Toys may represent the major source of childhood exposure to DINP because PVC plastics are often used in children's products, and DINP is currently the major plasticizer used in these products (Rastogi, 1998; Marin, 1998; CPSC, 1998; Health Canada, 1998). Other phthalates such as DEHP have been or are also used in a variety of products (Rastogi, 1998; Marin, 1998). U.S. toy manufacturers began voluntary removal of DEHP from pacifiers and nipples in 1986 (TMA, 1986). DEHP was the predominant plasticizer used in soft PVC children's products, but since the early 1980's, has been replaced in most countries by other plasticizers, in particular DINP (Steiner et al., 1998; Wilkinson and Lamb, 1999). At the request of CPSC, U.S. toy manufacturers and importers voluntarily stopped using DINP and other phthalates in

teethers, rattles, and bottle nipples (see Section I). The voluntary action, which became effective in March 1999, applies to products intended to be mouthed; it does not apply to other children's products, such as squeeze toys and rainwear. The scope of the action is similar to the voluntary standard for DEHP.

Table IV-1. Estimated U.S. end uses of DINP produced in 1998

End Use	**Subtotal (10^3 metric tons)**	**Total (10^3 metric tons)**
Film and Sheet		13
Stationary and Wood Veneer	6	
Pool Liners	1	
Other	6	
Flooring		48
Tiles	23	
Sheets	25	
Artificial leather		3
Coated Fabrics		21
Tarps	16	
Conveyor Belts	1	
Other	4	
Dip Coating/Slush Molded		30
Gloves	15	
Toys	6	
Traffic Cones	<1	
Other	~9	
Tubings and Profiles		7
Profiles	5	
Garden Hoses	2	
Wire and Cables		32
Shoes/Shoe Soles		9
Under-Body Coating		7
Sealants (carpet backing)		8
GRAND TOTAL		178

Source: NTP/CERHR, 2000

As noted above and reviewed previously by CPSC (1998), DINP is one of many DAPs used in different products made from PVC and other plastics, including vinyl flooring, building materials, automobile interiors, medical devices, and other consumer (e.g., children's) products. DINP is used in vinyl upholstery, wire and cable, coated fabrics,

footwear, and children's products as shown in Table IV-1 (Wilkinson, 1998; CPSC, 1998, NTP/CERHR, 2000). Other widely used DAPs (e.g., DEHP, DBP, and butyl benzyl phthalate) have been detected in food (ATSDR, 1993; MAFF, 1996a; Yin and Su, 1996; Giam and Wong, 1987), infant formula (at low non-quantifiable levels) (Baczynskyj, 1996; MAFF, 1996b), water (ATSDR, 1993; Yin and Su 1996), ambient air (ATSDR 1993), indoor air sedimented residential dust (Ølie et al., 1997), and soil (ATSDR, 1993), and from medical devices (Barry et al., 1989; Plonait et al., 1993). Other than consumer products, food is believed to be the primary source of exposure to DAPs (ATSDR, 1993). Because they are not generally used in food packaging in the U.S., DAPs present in food may occur though general environmental contamination (ATSDR, 1993; MAFF, 1996a-b), or food processing equipment.

A number of estimates for human intake of DAPs appear in the literature. Average exposure in the U.S. to DEHP was estimated to be about 3.8 $\mu g\ kg^{-1}d^{-1}$ (ATSDR, 1993). Typical total intake of DEHP in Canada was estimated to range from 8 to 19 $\mu g\ kg^{-1}d^{-1}$ for various age groups, with the greatest exposure in 0.5- to 4-year-old children (Meek and Chan, 1994). The average dietary intake of total DAPs in the U.K. was estimated to range from ~1 to 11 $\mu g\ kg^{-1}d^{-1}$ (MAFF, 1996a). An estimate of dietary intake of total phthalates by infants in Europe was reported to be 23 $\mu g\ kg^{-1}d^{-1}$ (Janssen et al., 1998). A study by the U.K. Ministry of Agriculture, Fisheries, and Food derived, from levels in unreconstituted infant formula, average DAP intakes via infant formula of 130 $\mu g\ kg^{-1}d^{-1}$ at birth and 100 $\mu g\ kg^{-1}d^{-1}$ at 6 months of age (MAFF 1996b). Lower DAP levels have been found in infant formula in the U.S.—namely, 0.011-0.051 μg/g in ready to use formula and 0.007-0.032 μg/g in powdered formula (Baczynski, 1996). These levels suggest intakes in the U.S. via infant formula of 0.05 to 5 $\mu g\ kg^{-1}d^{-1}$ in 5- to 12-month-old infants (CPSC, 1998). This is based on 125 g of powder per quart (1.14 L) of reconstituted formula (typical of manufacturers' instructions), 3-4 feedings/day at 210-240 mL/feeding (Nelson et al., 1996, p. 162), and a 10-kg body weight.

B. Migration from Toys

DINP content in plastic toys has been measured to be typically ~20 to 40%, but in some items more than 50%, of the dry weight (Table IV-2). For example, Chen (1998a) measured DINP in 31 of 35 products and found a concentration range of 15.1 to 54.4% dry weight. Of 41 children's products made in the U.S., China, and Thailand, Health Canada (1998) detected DINP in 27 (66%) in concentrations that ranged from 3.9 to 44 % dry weight. DEHP was detected at far lower concentrations in 24 of the products (1 of 5 from the U.S., 22 of 35 from China, and 1 of 2 from Thailand). Criteria for product selection were not discussed in the Health Canada (1998) surveys. An analysis of 15 samples of PVC materials used to manufacture toys in Spain revealed a mixture of plasticizers including DINP, DEHP, and DIDP, and reported DEHP contents ranging from <0.1 to 34% dry weight, with 6 of 15 samples containing >10% DEHP dry weight (Marin et al., 1998). DINP and DIDP were found in 4 of 4 teethers studied (~32 to 40% w/w), and in 2 of 3 dolls (~20 and 26%, respectively), studied by Rastogi (1998). This author also reported DEHP in 3 of 4 teethers (0.01 to 0.07% dry weight) and in 2 of 3

dolls (0.12 and 22.4% dry weight, respectively), as well as trace amounts of DBP, DEP, or BBP in some the items studied. More than a decade ago DEHP concentrations of approximately 30 to 42% dry weight were detected by Lay and Miller (1987) in U.S.-manufactured pacifiers. More recent data like those just summarized suggest that the Lay findings do not reflect DEHP concentrations in products intended to be mouthed that are currently manufactured in the U.S. and covered by the voluntary ban (TMA, 1986).

When children mouth toys, DINP and/or other plasticizers can migrate into saliva, and subsequently swallowed as well as dermally absorbed through the oral mucosa. Experiments undertaken to estimate the extent of DINP migration into saliva are summarized in Table IV-2 and below. They indicate that a substantial amount of DINP migration may take place, under certain, somewhat poorly defined conditions, given the variability in measured migration rates obtained to date. In its previous review, CPSC (1998) did not attempt to quantify potential DINP exposures from mouthing pacifiers because it was not believed that any pacifiers containing DINP were being marketed in the U.S. However, because there currently is no enforceable regulatory restriction on the use of DINP in domestically or foreign-made pacifiers sold in the U.S., potential exposures to DINP from mouthing pacifiers are also estimated. The estimates and their derivation are provided only as part of a supplementary analysis appended to this report (see Appendix A).

In vitro studies

To estimate migration of DINP from mouthing toys, CPSC undertook experiments using pneumatic piston impaction applied to samples cut from plastic toys and bathed in a simulated-saliva liquid. The method was intended to approximate the effects of child biting/chewing, similar to that used previously to estimate exposure to DEHP (CPSC, 1983). DINP migration from 31 DINP-positive children's products ranged from 1.0 to 48 μg per 11 cm^2 h^{-1} (Table IV-2) (CPSC, 1998). Neither increased piston force (from 6 to 12 pounds), nor increased piston area conditional on constant pressure, nor periodic replenishment of saliva simulant, significantly increased observed migration rates (Chen, 1998a).

Briefly, to obtain these results, the product tested was held in place in a stainless steel beaker and immersed in 50 mL saliva simulant composed of Dulbecco's phosphate buffered saline (PBS) at pH 7.2 supplemented with 0.16% mucin. The pH of the simulant is within the range of 5.5 to 7.5 (average 6.7) reported for human saliva (Afonsky, 1961). While the average mucin content of human saliva is 0.25% (Afonsky, 1961), previous experiments with DEHP-containing products showed that migration rates obtained with 0.16% mucin (in PBS or Hank's balanced salt solution) more closely match those obtained with adult human saliva (CPSC, 1983). Intact products were used if possible or otherwise were cut to a size small enough to fit in the beaker. A hexagonal pneumatic piston with a surface area of 2.18 cm^2 impacted the sample for 6 h at a rate of 15× min^{-1} with a force of 6 pounds (27 Newtons). A subset of products was also extracted by gentle shaking without piston impaction. Extracts were analyzed for DAPs by gas chromatography-mass spectroscopy. Measured migration rates with and without

Table IV-2. Experimentally measured DINP migration from PVC children's products

Study	Products	DINP content (%)	No. tested	Method	Units	Mean	SD	Range (Min - Max)	Mean in μg/11 cm^2/h	Reference
Laboratory										
CPSC	Teethers, toys	15.1 - 54.4	31	Impaction	μg/11 cm^2/h	8.2	9.83	1.0-48.1	8.2	Chen (1998a)
Health Canada	Teethers, toys, pacifiers	3.9 - 44	27	Impaction [a]	μg/10 cm^2/h	0.32	0.08	NR [b]	0.35	Health Canada (1998)
Austrian Standards Institute (ASI)	Teether	36	1	Static	μg/dm^2/h [c]	12.7	NR	NR	1.4	Fiala et al. (2000)
				Shaking	μg/dm^2/h	36.3	NR	NR	4.0	
				Ultrasound	μg/dm^2/h	387.3	NR	NR	42.6	
Danish Environmental Agency	Teethers	NR	2	Shaking	μg/g or ppm	NR	NR	89-24,691	NA	Vikelsøe et al. (1997)
					μg/dm^2/h [d]	10,923	10,102	54 – 23,260	1,202	Rastogi et al. (1997)
TNO [e]	Toys, teethers	21.0 - 46.6	10	Head-over-heels	μg/10 cm^2/min	2.4	1.38	0.9-5.6	158	Rijk and Ehlert (1999)
LGC [f]	Teether, toy	NR	2	Shaking 37°C	μg/10 cm^2/min	0.95	0.35	0.7-1.2	63	Axford et al. (1999)
				Shaking 65°C	μg/10 cm^2/min	4.5	0.78	3.9-5.0	294	
Human Subjects										
CPSC	Toy (disk) [g]	NR	5	Mouthing	μg/10.3 cm^2/h	246.8	94.7	160-384	263.6	Chen (1998a)
ASI	Teether	36	1 [h]	Sucking	μg/dm^2/h [c]	833	NR	NR	91.6	Fiala et al. (2000)
		36	1	Chewing	μg/dm^2/h	1330	NR	NR	146.3	
Dutch consensus group	Standard disk, teether [i]	NR	3		μg/10 cm^2/min	1.8	NR	0.4-2.4	118.8	RIVM (1998)

[a] Impaction was with a "bite form" used to test the resistance of toys to breaking. Units were micrograms per 10 square centimeters per hour.
[b] NR, not reported. NA, not available.
[c] Original units were micrograms per square decimeter, for either 1 or 3 hours. All values shown here were adjusted to 1 hour
[d] Units were in micrograms per square decimeter per day.
[e] This was an interlaboratory study coordinated by the TNO Nutrition and Food Research Institute. Units were micrograms per 10 square centimeters per minute.
[f] This was an interlaboratory study coordinated by the Laboratory of the Government Chemist. In this method, glass balls are added to the flask to aid extraction.
[g] 20 disks were cut from 5 identical toys. Ten disks were tested by 10 subjects (the remaining 10 were tested by impaction). Units were micrograms per 10.3 square centimeters per hour.
[h] 9 human subjects participated.
[i] Test articles included disks cut from a specially prepared PVC sheet, a teether, and disks cut from the same type of teether.

the piston were used to estimate migration fractions due to diffusion (which depends on sample surface area) and to piston action as previously described (Chen, 1998a; cf. also CPSC, 1983). This information was used to calculate the migration rate in μg h^{-1} for a product-portion size (11 cm^2) able to fit in a child's mouth (as in CPSC, 1983).

As previously reviewed (CPSC, 1998), other laboratory methods of measuring DAP migration (e.g., shaking, ultrasound, and impaction) led to a broad range of results (Rastogi et al., 1997; RIVM, 1998; Earls et al., 1998; Steiner et al., 1998). Ultrasound generally produced greater migration rates, and a variety of agitation methods have produced migration rates both less than and greater than the impaction method used by CPSC (1998). The limited data currently available from different laboratories continues to make direct comparisons among methods difficult. The migration rate (equivalent to 2,560 μg per 11 cm^2 per h) reported by the Danish National Environmental Research Institute using a shaking method applied to a disk cut from a particular toy (Rastogi et al., 1997) could not be replicated when CPSC staff applied the same method to the same toy. In the hands of CPSC staff the test resulted in somewhat lower migration estimates than those produced using the CPSC impaction method (Chen, 1998b).

Notably, no significant positive correlation has been reported between DINP concentrations in children's PVC toys and measured rates of DINP migration from those toys (CPSC, 1998; Health Canada, 1998).

In vivo ("chew and spit") studies

Extractions of DEHP and DINP have also been studied *in vivo* using adult volunteers as surrogates for children. Such studies have been performed in parallel with *in vitro* studies allowing a direct comparison of *in vivo* to *in vitro* results (Table IV-2). For example, Steiner et al. (1998) measured migration of DEHP into a saliva simulant using a standardized PVC film subjected to static vs. dynamic conditions (shaking with glass balls/plates, vs. simulated chewing with glass dentures). They also performed three 3-hour and two 6-hour sucking tests using a single adult volunteer from whom all saliva was collected. Extractions varied ~40-fold among the various experimental scenarios. Adult sucking was found to be comparable to static *in vitro* extraction methods studied which yielded the lowest DEHP migrations (~40 to 60 μg DEHP per dm^2 film).

In a study reported by the Dutch Consensus Group (RIVM, 1998) salivary extraction of DINP was measured from 10-cm^2 test specimens containing DINP at concentrations: 38% (specimen 1), 43% (specimen 2), and 43% (specimen 3), as well as a control specimen without DINP. Specimens 2 and 3 were differently shaped parts derived from the same commercially available teething ring. A total of 20 volunteers were instructed to suck and bite on the control specimen for 10 to 15 min during which all saliva was collected, and then to rest for 5 min. This procedure was repeated three times using the same piece of test specimen 1, resting 5 min between each session. This procedure was then repeated with 10 volunteers using test specimen 2 and with the other 10 using test specimen 3. The mean (and range) of DINP extractions from specimens 1-3 were approximately 1.4 (0.3-8.3), 2.4 (0.9-8.9), and 1.6 (0.9-5.7) μg min^{-1}, respectively, with

the overall mean across all groups being 1.8 μg min^{-1} (RIVM, 1998), which is equivalent to 120 μg per 11cm^2 per h. There was no correlation between extraction and pH or protein content of the saliva, and release rates over the various 15-min intervals seemed consistent (RIVM, 1998).

Extractions of DINP were measured by Chen (1998a) using five pairs of adults who mouthed 10.3-cm^2 plastic disks cut from five identical toy ducks. These *in vivo* extractions were compared by CPSC (1998) to the corresponding *in vitro* extraction measurements made by applying the piston impaction method to similarly sized disks cut from the same toys (see *in vitro* subsection above). The methods used were essentially the same as those used previously by CPSC (1983) to study DEHP extraction from toys.

In contrast to results obtained by Steiner et al. (1998) for DEHP, the five ratios of *in vivo* to *in vitro* DINP extraction for the toys measured by Chen (1998a) ranged from approximately 23 to 73, with a geometric mean (GM) and geometric standard deviation (GSD) of 39.5 and 1.58, respectively (CPSC, 1998). A corresponding "adjusted" GSD of approximately 1.92 would be required to reflect uncertainty associated with estimating the mean and variance of the logs of the five reported ratios. This estimate is based on Monte-Carlo simulation of the corresponding doubly compounded normal distribution with a t- and Chi-square-distributed mean and variance with 9 and 4 degrees of freedom, respectively. The average (±1 SD) of the five means of paired corresponding measured DINP-extraction rates was 246.8 ± 94.7 μg per 10.3 cm^2 h^{-1} (24.0 ± 9.19 μg cm^{-2} h^{-1}), with a GM of 233.4 μg per 10.3 cm^2 h^{-1} (22.7 μg cm^{-2} h^{-1}) and a GSD of 1.45 (Table IX-2). Accounting as above for uncertainty associated with estimating the mean and variance of the logs of the five reported mean DINP extraction rates, the "adjusted" GSD would be 1.70 (e.g., implying corresponding 1-tailed upper 95% and 99% DINP-extraction confidence levels of about 60 and 100 μg cm^{-2} h^{-1}, respectively).

Toy-mouthing behavior among children

A substantial amount of mouthing in infants and young children less than 3 years of age is expected as part of normal early childhood developmental behavior. Recent detailed studies of the extent of time young children spend mouthing toys provide an empirical basis for population-wide estimates.

Zartarian et al. (1998) videotaped mouthing behavior of two Mexican-American farm-worker children of age 2 years and two of age 4 years for a single day, and found that they mouthed non-dietary objects for total durations of approximately 7, 28, 16, and 22 min, respectively. The totals comprised multiple contributions of very short (~9- to 13-second) duration. Objects most frequently mouthed included skin (primarily hands), hard toys, and hard surfaces. In addition, one child frequently mouthed photographs on the day of observation.

A 2-day parent-observation study was performed by the Dutch Consensus Group using 42 children aged 3–36 months (Groot et al., 1998). In this study, mouthing times were derived from 2.5 h of logged parent observations of each child. Mouthing time was

calculated for the time children were awake but not eating, during ten 15-min observation periods over 2 days. Logs were kept of which objects were mouthed, and these objects were divided into ones intended vs. not intended for mouthing.

Table IV-3. Extrapolated daily mouthing time for all objects observed to be mouthed in the Dutch Consensus Group study, excluding pacifiers*

Age (months)	*n*	Mean (min)	Standard Deviation (min)	Minimum (min)	Maximum (min)
3-6	5	36.9	19.1	14.5	67.0
6-12	14	44.0	44.7	2.4	172.
12-18	12	16.4	18.2	0	53.2
18-36	11	9.3	9.8	0	30.9

*Adapted from Groot et al. (1998)

Groot et al. (1998) found no differences between results from separate days for each child, and so combined the daily estimates for each child by age category. Using a Kruskal-Wallis test, they reported a significant decreasing trend in mouthing duration with increasing age. Results for non-pacifier objects for each age group were extrapolated to a 24-hour period, as listed in Table IV-3.

A more recent study involved participants drawn (roughly equally) from a consumer-feedback database of local families maintained by the Fisher-Price Child Research Center Play Laboratory and the East Aurora Community Nursery School and Day Care Center, both in East Aurora, NY (Juberg et al., 2001). Additional demographic characteristics of the participants in this study were not provided. This study relied on standard diary forms with instructions for participating parents to observe their children in a normal environment (primarily home), and to document both the type and duration of each item mouthed (Juberg et al., 2001). One-day observations were first performed on groups of children between the ages of 0-18 months (n=107, approximately uniformly distributed in age between ~4.5 and 18 months) and 19-36 months (n=110, age distribution not provided), including observations of pacifier usage for both age groups. A final study phase involved observations for 5 non-consecutive days over a 2-month period on 168 children between the ages of 3-18 months at study initiation, focusing on total time mouthing objects other than pacifiers. Results obtained from the initial one-day studies are summarized in Table IV-4.

Table IV-4. Summary of average mouthing durations observed during 1-day observation periods in the study by Juberg et al. (2001)[a]

Age group	Object type	All participants		Only those who mouthed object(s)	
		n	Mean ±1 SD (min)	*n*	Mean (min)
0-18 months	Pacifier	107	108 ± 187	52	221
	Teether	107	6	34	20
	Plastic toy	107	17	66	28
	Other objects	107	8	46	22
	Non-pacifier	107	33 ± 46	–[b]	–
19-36 months	Pacifier	110	126 ± 246	52	462
	Teether	110	0[c]	1	30
	Plastic toy	110	2	28	11
	Other objects	110	2	18	15
	Non-pacifier	110	5 ± 14	–	–

[a] Adapted from Juberg et al. (2001).
[b] Value of "–" signifies "not reported".
[c] Only one child in the 19-36 month group used a teether, for a reported duration of 30 min.

The data reported indicate that a substantial fraction of the children observed did not engage in mouthing activity, and that mouthing-time distributions for the remainder were highly skewed. For example, reported pacifier-mouthing times for children of age 0 to 18 months, and of age 19-36 months, included several mouthing durations between 10 and 15 hours. Eight infants in the former group were reported to use pacifiers "throughout the night" such that "the exact mouthing time for these infants is not known" (Juberg et al., 2001). Likewise, mouthing times for non-pacifiers ranged up to 320 and 100 minutes among children 0-18 months and 19-36 months old, respectively. Notably, the mean durations of pacifier-mouthing times among younger (0-18 month-old) and older (19-36-month-old) children who did mouth pacifiers were ~2.0- and ~3.7-fold greater than those of all children in those two age groups, respectively. No significant correlation was found between mouthing duration of pacifiers and that of non-pacifier objects (Juberg et al., 2001).

The distribution of daily mouthing times for pacifiers observed among 110 children 19-36 months of age observed in the study by Juberg et al. (2001) is shown in Figure IV-1 (Juberg et al., 2001). This figure shows that the distribution of pacifier mouthing times is bimodal, with a substantial fraction of children mouthing pacifiers from 0 to 100 minutes,

and with a smaller yet substantial subset of children mouthing pacifiers from 300 to 800 min (i.e., from 5 to ~13 h d^{-1}, with a modal mouthing duration of 10 h d^{-1}).

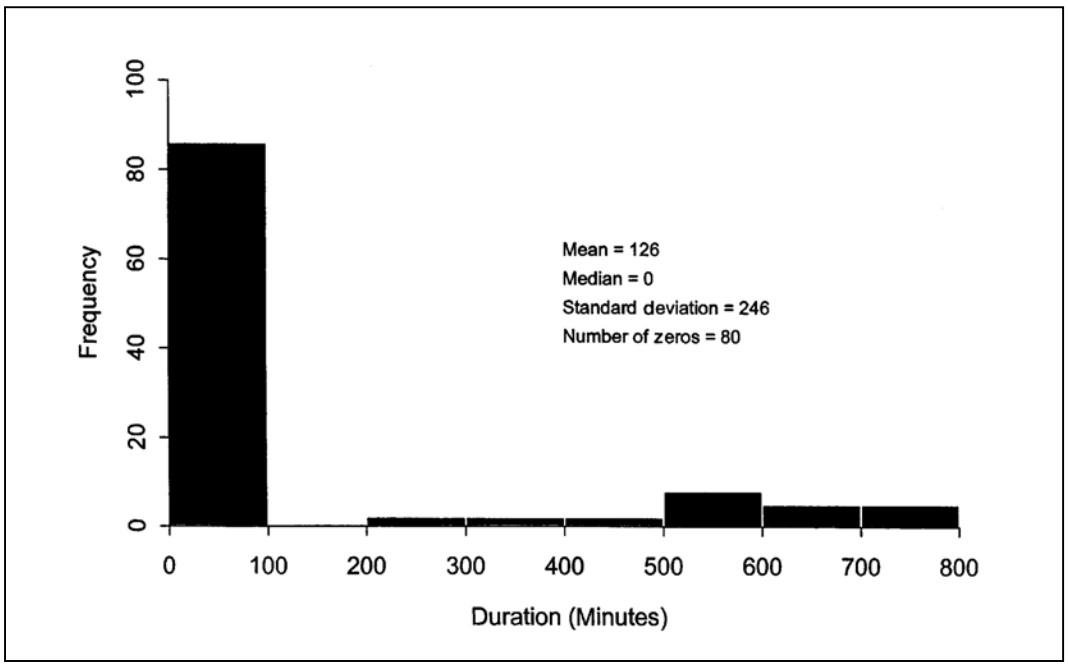

Figure IV-1. Distribution of daily mouthing durations for pacifiers (including teethers and toys) among 110 children 19-36 months; reprinted from Juberg et al. (2001) with permission

The distribution of 5-day average mouthing times for non-pacifiers (including teethers and toys) among 168 children 3-18 months old at recruitment (4-21 months old during the observation period) is shown in Figure IV-2. Several of the 168 children observed for 5 days consistently mouthed objects for more than 2 hours a day. Linear regression for children approximately 4.5 to 20 months of age indicated the following significant negative relation between average mouthing duration t_m (min) and child age a (months): $\log_e a \approx 4.5 - t_m/8$ ($R^2 = 0.15$, $p < 0.001$) (Juberg et al., 2001). For the 0-18 month-old age group, the mean non-pacifier mouthing duration of the 107 one-day participants (33 min) was not significantly different from that of the 168 5-day participants (36 min) (p=0.47 by T-test) (Juberg et al., 2001). With regard to the non-pacifier mouthing-time distributions for this group, Juberg et al. (2001) comment that the 5-day average for individuals (n=168) vs. combined daily mouthing times (n=810) revealed "fewer zeros and a lower maximum value … for the 5-day average … than for the daily observations." However, the standard deviations (48 vs. 52 min) for these two groups of data do not differ significantly ($F_{809,167} = 1.17$, 2-tail p = 0.20), indicating that relatively little of the variability associated with the 810 combined daily mouthing times is attributable to day-to-day variations in an individual's mouthing time.

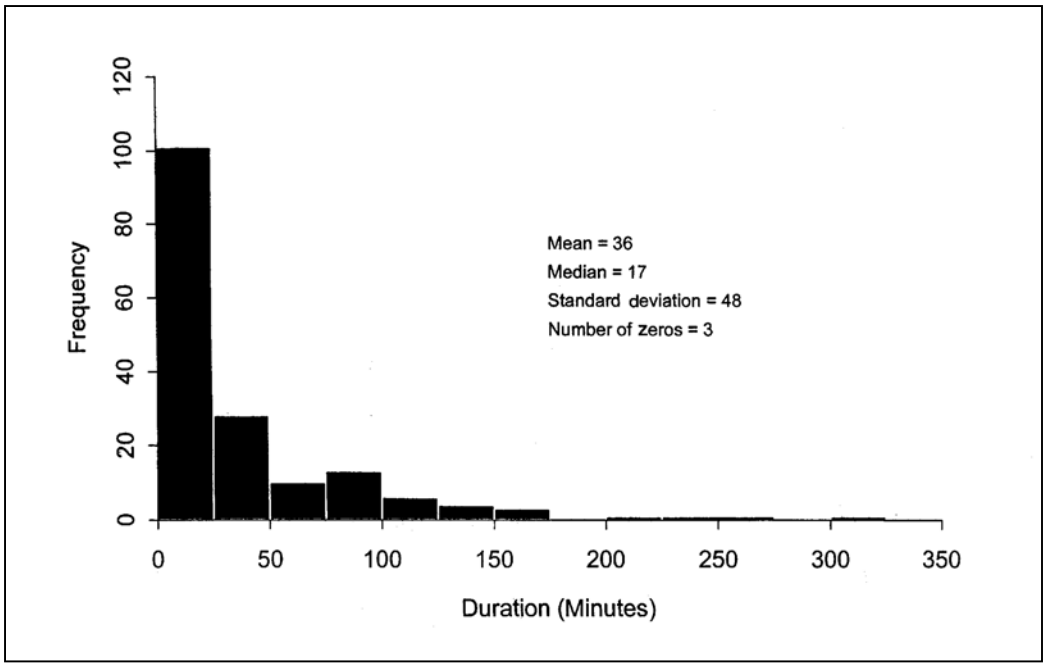

Figure IV-2. Distribution of 5-day average mouthing durations for non-pacifiers (including teethers and toys) among 168 children 3-18 months old at recruitment (4-21 months old during observation); reprinted from Juberg et al. (2001) with permission.

Results of available juvenile-mouthing studies indicate that mouthing behavior is common and routinely engaged in during childhood, and is dependent both on the age of the child and items mouthed. Mouthing duration varies considerably among children, with some consistently not mouthing any objects, most mouthing plastic objects for less than an hour a day, and with a small yet non-negligible fraction (1% to 5% of children) mouthing objects for more than two hours a day, for averaging periods of at least 5 days. The studies also reveal a wide range in the types of objects mouthed, including many non-toy objects containing flexible plastic, such as electrical extension cords, plastic bags, and telephone cords (see Table 2 of Juberg et al., 2001). Both the Groot et al. (1998) and Juberg et al. (2001) studies show that: 1) young children mouth pacifiers for significantly longer durations than other objects, 2) a subset of children 0-36 months of age mouth pacifiers for about 10 h d^{-1}, and 3) on average, mouthing times for all non-pacifier objects are significantly greater among children 0-18 months compared to those 19-36 months old. Juberg et al. (2001) recorded pacifier mouthing times $\geq$10 h d^{-1} for a number of children observed in both age groups studied—i.e., mouthing times indicating that these children were mouthing pacifiers for nearly the entire time they were awake every day, and in the case of some infants, for an unknown amount of additional time at night as well.

A "Phthalate Observational Study and Telephone Survey" is currently being undertaken by CPSC staff to further investigate potential exposure and health risks to children under six years of age from PVC teethers, rattles, and other toys that contain one or more DAP plasticizer. Based on its previous study, the CPSC (1998) concluded that few, if any,

children are at risk of liver or other organ toxicity from mouthing PVC toys that contain DINP. The new CPSC study seeks to reduce residual uncertainties associated with its previous assessment, particularly regarding the types of toys that children are mouthing and how long they typically mouth these toys. To further examine the range of DAP exposures in various age groups, an observational study is underway in which children are being observed in their natural settings (e.g., home, babysitter, childcare) during two half-day (three-hour) sessions by trained non-parental observers. A telephone survey is being used to recruit subjects for the observational study as well as to determine whether the proportion of children affected and the intensity of exposure to phthalates is significant enough to merit further studies of children in the 3 to 6 age group.

The CPSC study is taking place within two geographical areas (Chicago, IL and Houston, TX). About 100 children from 3 through 35 months old will be observed in each study area, with about 40 children aged 3 to 11 months, 30 children from 12 to 23 months, and 30 children from 24 to 35 months old. The only formal inclusion criteria for subjects are age and area, although gender, income, race and child-care groups are being monitored for each child recruited. If the samples show substantial under-representation of any of these groups, additional quota sampling requirements may be imposed. Children meeting the inclusion criteria are being identified via random-digit dialing (RDD) techniques in each study area.

For 20 minutes out of each half-hour, each child's mouthing activities are being recorded quantitatively, alternating (randomly) with a 10 minute period during which the observer records these activities by writing into a diary. For the purpose of this study, mouthing is defined as placing any item to/into the child's lips, tongue, or mouth for any length of time. The item being mouthed, as well as the type and stopwatch-timed duration of mouthing activity (e.g., sucking, chewing, touching the object to the lips) is being recorded.

DINP exposure levels due to childhood mouthing of consumer products

Both CPSC and the Dutch Consensus Group estimated daily DINP intakes based upon extraction rates and toy-mouthing observations from the Dutch study described above. Using Monte-Carlo procedures and a variety of assumptions to model DINP exposure, CPSC (1998) estimated that the 95th percentile of exposure among 3- to 12-month olds is about 94 μg kg^{-1} d^{-1} with a corresponding 95% confidence interval (CI) with respect to estimation error of ~50 to 230 μg kg^{-1}d^{-1}. For 13- to 26-month olds the estimated 95th percentile (and corresponding 95% CI) is 7.6 (4.4-17) μg kg^{-1} d^{-1} (Table IV-5).

The Dutch Consensus Group used Monte Carlo simulation and estimates of mouthing time and the leaching rates from its *in vivo* study of 20 adults to obtain 95th percentiles of exposure among 3-12, 12-18, and 18-36 month-olds of ~26, 10, and 4.3 μg kg^{-1} d^{-1}, respectively, without specifying associated CI values (Table IV-5). Using the data on the three samples in the Dutch study (RIVM 1998), Health Canada (1998) estimated 95th and 99th percentile exposures for 3- to 6-month olds of ~74 and ~174 μg kg^{-1} d^{-1}, respectively, without specifying associated CI values (Table IV-5).

Table IV-5. Estimated exposure to DINP in children's products (micrograms $kg^{-1}d^{-1}$)

Agency	Product(s)	Age	Mean	Median	95th %-tile	Range[a]	Reference
CPSC[b]	Teethers, toys	3-12 months	5.7	NR[c]	94.3	NR	CPSC (1998)
		13-26 months	0.7	NR	7.6	NR	
Health Canada	Teethers, toys	3-12 months	44	NR	NR	4-320	Health Canada (1998)
		13-26 months	39	NR	NR	5-228	
	Pacifiers	3-12 months	120	NR	NR	18-640	
		13-26 months	62	NR	NR	5-458	
Austrian Standards Institute	Teethers	NR	31.25	NR	NR	NR	Fiala et al. (2000)
Dutch consensus group[d]	Teethers	3-6 months	9.66	7.17	26	NR-70.7	RIVM (1998)
		6-12 months	7.79	4.8	25.5	NR-142	
		12-18 months	2.33	1.06	10.5	NR-51.1	
		18-36 months	1.13	0.521	4.32	NR-23	

[a] Lower bound provided is the minimum, upper bound the maximum
[b] To derive the estimate, the migration rate by the impaction method was multiplied by a scaling factor to adjust for the difference between extraction via impaction *in vitro* and observed *in vivo* using human subjects.
[c] NR is not reported
[d] Based on the migration rates measured with human subjects by the Dutch consensus group.

Differences among the estimates presented in Table IV-5 highlight uncertainties inherent in DINP-exposure calculations due to: (1) lack of correlation between DINP extraction and DINP content, (2) substantial variability in DINP extraction across laboratory procedures as well as human subjects, and (3) the unknown number and distribution of children's products containing DINP. No discussion of developmental age, physical condition, ethnicity, or other sociodemographic indicators of study subjects is provided in the largest available studies.

Estimated Oral-Ingestion Exposures to DINP from Children's Toys

Plausible upper bounds on the extent of potential DINP exposure from children's toys may be estimated using the more recent information summarized above. Non-pacifier toys were observed by Juberg et al. (2001) to be used for approximately 3 h d^{-1} by subsets comprising more than one child among 107 children 0-18 months of age, and for approximately 1 h d^{-1} by subsets comprising more than one child among 110 children 19-36 months old. As mentioned above, the 95% upper confidence bound on the CPSC estimated DINP extraction from children's toys based on *in vivo* data is ~60 μg cm^{-2} h^{-1}. Assuming child body weights of approximately 7 and 10 kg for ages 0-18 and 19-36

months, respectively, corresponding daily mouthing times of 3 and 1 h d^{-1}, and a non-pacifier-toy surface area of 11 cm^2, corresponding daily DINP exposures to relatively highly exposed children 0-18 and 19-36 months old would be approximately 280 and 66 μg kg d^{-1}, respectively.

C. Potential Dermal Exposure to DINP that May be in Consumer Products

Dermal exposures to DAPs are expected to occur due to contact of external skin and/or oral mucosa with flexible plastic products containing one or more DAP plasticizers (CPSC, 1985). The most significant dermal exposures to DAPs from consumer products are expected to involve those products for which the duration of regular dermal contact is longest, particularly children's pacifiers (if applicable), mouthed children's toys, and bedding and clothing articles made with PVC. Thus, PVC playpens, rainwear (e.g., pants, coats, ponchos, hats), plastic dancing/fashion clothing, footwear (e.g., rain shoes, boots, shoes, shoe/sneaker insoles, slippers, sandals including "jelly sandals", and thongs), gloves, and jewelry are potential sources of significant dermal exposure to DAPs. DINP exposure could results from exposure to any of these PVC products that are plasticized with DINP. DINP exposure may thus occur among children or adults who wear PVC footwear, including PVC shoes or "jelly" sandals (see Figure IV-3), insoles or arch supports plasticized using DINP. Exposure would be increased when these products are worn routinely without socks, as may be expected throughout the year in warmer U.S. climates, and during warm seasons or indoors in all U.S. climates.

Data on national usage and contact rates for such consumer products, together with corresponding concentrations of specific phthalate ester plasticizers (if applicable), are currently unavailable. For most such products, concentrations of DINP and other plasticizers are not usually supplied to consumers by retailers, are often not known by suppliers, and are rarely measured by the CPSC or any other regulatory agency. Even manufacturers of such injection-moulded products rarely have this information, because they typically obtain their PVC injection-moulding material as stock or custom-made compounds (e.g., pre-plasticized PVC pellets) purchased from "compounder" companies. These companies generally fabricate and deliver PVC compounds to consumer-product manufacturers without information concerning the amounts and identities of specific plasticizers contained in these compounds.

At the request of the CHAP, the CPSC chemistry laboratory tested two samples of PVC "jelly" sandals (Chen, 2000). Neither sample was found to contain DINP. One sample of yellow PVC jelly sandals purchased in California was determined to contain 13.5% DEHP and 15.7% diisobutyl/dibutyl phthalates (total phthalate content 29.2%). The second sample was purchased in Maryland and had a pink sole with clear straps; the sole was determined to contain 31.8% DEHP, and the straps were found to contain 31.6% DEHP and 2.5% dibutyl phthalate (total phthalate content 34.1%). In terms of either total phthalate content or the absence of DINP, it is unknown how representative these two CPSC measurements are of PVC sandals currently available in U.S. markets, many of which are currently imported from East Asia, Brazil and Mexico. The two CPSC

measurements clearly indicate, however, that some PVC sandals contain considerable amounts of plasticizers, including DEHP. In the manufacture of PVC consumer products with limited expected lifetimes (e.g., "jelly" sandals), DEHP would typically be preferred as a plasticizer over DINP or higher molecular-weight phthalate diesters because DEHP is marginally less expensive and slightly more cost-effective than competing plasticizers, and can be used to induce similar plasticity properties with a greater product lifetime (ExxonMobil, 2001).

It is reasonable to expect that the largest time-weighted average exposures to DAPs (in mg $kg^{-1}d^{-1}$) occur during early childhood (e.g., 0-36 months of age). Activities in later childhood and among adults are much more varied than those during early childhood, a large number of PVC products designed for young children potentially contain DAPs, and young children weigh considerably less than older children and adults. Based on measures of DEHP migration rate into lanolin and estimates of daily contact with various children's items, such as playpens and vinyl baby pants, the CPSC previously estimated a collective yearly exposure to these two items of 216 mg (96 mg from playpen contact + 120 mg from wearing rubber pants, assuming 10% absorption efficiencies) (CPSC, 1983). Procedures CPSC used to calculate this exposure range were critiqued by Rodricks and Turnbull (1984), who concluded that some of the assumptions used by CPSC were likely to overestimate human exposure. The 1985 CHAP report (CPSC, 1985) responded that the CPSC estimates of DEHP exposure from children's items were reasonable in view of available data.

In comparison to DINP, DEHP has a slightly lower molecular weight (390 vs. 418, respectively), and an apparently greater estimated log octanol:water partition coefficient ($\log_{10}K_{OW} \approx 9.64$; (Leyder, 1983) vs. $\log_{10}K_{OW} \approx 9$; see Appendix, respectively). This suggests that the dermal absorption of DINP would be somewhat less than, and certainly no greater than, that of DEHP (see Appendix A). However, few data are available upon which to base a confident prediction of percutaneous DINP uptake by human skin in the context of repeated, sustained, humid, and non-static contact with DINP-plasticized PVC plastic. Likewise, no studies have measured percutaneous absorption of DINP through the oral mucosa. Both for external skin and oral mucosa, some degree of percutaneous DINP is expected based on both available data and relevant theoretical considerations, which are discussed in Appendix A.

Figure IV-3. Examples of polyvinyl chloride injection-moulded "jelly" sandals currently marketed to U.S. infants, toddlers and young children. These products generally (but not necessarily) contain one or more dialkyl phthalate-ester plasticizers. This class of product typically contains diethylhexyl phthalate, but other plasticizers may used in addition and/or instead, such as dibutyl phthalate, diisobutyl phthalate, and possibly (but currently rarely) DINP. The specific products shown in this figure are intended only to illustrate the product class, and are not likely to contain any DINP.

D. Alternative Materials

Rigid polymeric plastics such as PVC require the addition of one or more plasticizers or other additives to induce flexibility and other properties that are desired in a flexible plastic product. Plasticizers are high-boiling point organic solvents used to impart flexibility to otherwise hard or brittle polymeric materials. A number of plasticizers besides DINP used for this purpose with PVC or other polymers are listed in Table IV-6, together with corresponding no-observed-adverse-effect levels (NOAEL) and comparative exposure data for certain compounds. Plasticizers generally cause a reduction in cohesive intermolecular forces along polymer chains resulting in various changed properties including reduced tensile strength, increased elongation, and reduced glass-transition or softening temperature. They also possess solvent power to insure polymer compatibility. Plasticizers are found in numerous flexible polyvinyl products such as wire insulation, electrical components, flooring, medical tubing, moldings, food film, refrigerator gasketing, low-fogging automotive components, emulsion paint systems, paper and textile coatings, and adhesives, in addition to children's toys and other consumer products discussed above.

While PVC use in toy production is limited, its use has been predominant in the manufacture of certain types of flexible toys (e.g., sheets for inflatable toys and doll's heads). Because of its brittle nature and heat sensitivity, PVC uses the greatest amount of additives of any commercial resin. Because of its susceptibility to dehydrochlorination, the vast majority of all stabilizers are used in PVC, as are 90% of all plasticizers and 95% of all phthalates (~1.4 billion pounds y^{-1}) used worldwide (Tickner, 1999). Alternative products (reviewed by Tickner, 1999) can also be processed using common processing techniques, some of which involve equipment identical to that already used to make PVC toys. These alternative plastics include: (1) thermoplastic elastomers, such as styrenic block copolymers, polyolefin blends, and elastomeric alloys; (2) ethylene vinyl acetate (EVA); and (3) polyolefins, such as very low density polyethylene (VLDP), linear low density polyethylene (LLDP), ultra-low density polyethylene (ULDP), and metallocenes such as syndiotactic polypropylene (sPP). Any one of these alternative plastics does not involve the large quantity of additives required to soften PVC. Clarity, low-temperature flexibility, heat sealability, impact strength, and durability of EVA, for example, are all improved by increasing its vinyl acetate content without using leachable plasticizers or other additives. Materials like EVA and sPP allow the use of simple joining techniques like radio frequency (RF) welding, and so represent useful substitutes to plasticized PVC sheets (Tickner, 1999).

Included among the plasticizers listed in Table IV-6 that are compatible with flexible PVC are ones that are not phthalates but that are high-solvating, polar, monomeric plasticizers. Diethyleneglycol dibenzoate, in particular, is claimed to be processed quicker and/or at lower temperatures than are vinyls made with phthalate plasticizers, to be resistant to extraction from PVC by aliphatic or oily solvents, and to be used extensively in applications ranging from floor tile to rotation-molded children's toys (Velsicol Chemical Corporation, 2000). This plasticizer was also recently shown to be nonmutagenic, nonclastogenic, and nonestrogenic in a battery of short-term tests, and to be relatively nontoxic in subchronic toxicity tests which indicated a NOAEL of 1000 mg $kg^{-1}d^{-1}$, with all treatment-related effects observed at higher subchronic doses apparently reversible (Smith et al., 2000).

A review of potential toxicity associated with non-DINP plasticizers, and with alternative plastics that do not require plasticizers such as DINP, is beyond the scope of this report. Decisions to remove DINP from consumer products due to any anticipated DINP-related toxicity should involve comparative assessments of health risks posed by the alternative DINP-free product. Based on information summarized in Table IV-6, DINP is relatively potent among those plasticizers examined to date in terms of chronic toxicity, with DBP and DEHP being only slightly more potent than DINP for identified chronic endpoints.

Table IV-6. Plasticizers used in consumer products

Plasticizer	Acro-nym	Critical toxic effect	NOAEL[a] (mg $kg^{-1}d^{-1}$)	Est. intake[a] (% of DINP)
o-Acetyltributyl citrate	ATBC	Decreased body weight	100	100
Acetyltriethyl citrate	ATEC	None identified	≥4,000	
Butylbenzyl phthalate	BBP	Decreased spermatozoal conc.	20	0.48
Dibenzothiazole disulfide[b]	DM			
Dibutyl maleate	DBM			
Dibutyl phthalate	DBP	Reduced F2 pup weights	5.2[c]	0.20
Diethyleneglycol dibenzoate[d] (Benzoflex® 2-45, K-FLEX®DE)	DEG-DB			
Di(2-ethylhexyl)adipate	DEHA	Fetotoxicity	30	39[e]
Di(2-ethylhexyl)phthalate	DEHP	Testicular damage	3.7	100
Di(2-ethylhexyl)maleate[f]	DEHM			
Diethyl oxalate	DEO			
Diisobutyl phthalate	DIBP			
Diisodecyl adipate	DIDA			
Diisodecyl phthalate	DIDP			
Diisononyl adipate	DINA	Increased liver weight	25	8.8
Diisononyl phthalate	DINP	Spongiosis hepatis	12-15	100
Diisooctyl phthalate	DIOP			
Diisopropylphthalate	DIPRP			
Dioctyl nylonate	DON			
Di-*n*-octyl phthalate	DNOP	Microscopic liver/thyroid changes	37	48
Dioctyl sebacate	DOS			
Dioctyl terephthalate	DOTP			
Dioctyl azelate	DOZ			
Diundecyl phthalate	DUP			
Dioctyl adipate	DOA			
Dipropylphthalate	DPRP			
(2-Ethylhexyl)acetate	2EHAc			
Mixed alkyl phthalate	MAP			
Mixed normal alkyl phthalate	MNAP			
Tributyl citrate	TBC	None identified	≥20,000	
Triethyl citrate	TEC	None identified	≥4,000	60[e]
Triisodecyl trimellitate	TIDTM			
Trioctyl trimellitate[g]	TOTM			
Mixed normal alkyl trimellitate[g]	TM			

[a] Adapted from CSTEE (1998, 1999). Estimated human exposures (expressed as a percent of estimated DINP intake) are only from mouthed toys, unless noted otherwise.

[b] Used in rubber shoes and rubber cloth.

[c] LOAEL was divided by 10 to estimate a corresponding NOAEL.

[d] Nonphthalate, high-solvating, polar, monomeric plasticizer having "excellent" compatibility with polyvinyl acetate (PVA) homopolymer and copolymer emulsions; also compatible with flexible PVC.

[e] From food-packaging materials.

[f] Unsaturated reactive ester that readily copolymerizes with vinyl acetate, vinyl chloride, acrylates and styrene; in copolymers, DEHM improves humidity and UV light performance.

[g] Plasticizers for polyvinyl chloride resin and copolymer applications.

E. Conclusions

Based on the physicochemical characteristics of DINP and limited monitoring data, the CHAP believes that general environmental exposure to DINP in the U.S. adult population is likely to be substantially lower than exposure to DEHP, which has been estimated at 0.003-0.030 mg $kg^{-1}d^{-1}$ (Doull et al., 1998). The most significant exposures to DINP are likely to occur from the use of consumer items that consist of or include flexible plastic that is plasticized using DINP. These consumer items currently include PVC teethers and other toys routinely mouthed by young children. Oral intake of DINP from pacifiers is not currently expected due to its withdrawal from these products by voluntary industry agreements with the U.S. CPSC (see Section A). To the extent these agreements remain voluntary and subject to violation at any time without notice to the CPSC, the magnitude of potential exposures to DINP and from pacifiers is addressed in the Appendix A.

Studies reviewed indicate that mouthing behavior among children 0-36 months old is dependent on child age and the types of items mouthed. Duration of mouthing varies among children, with some consistently not mouthing any objects and with a relatively small subset mouthing objects from 6 to 10 h d^{-1}. The studies also revealed wide variability in the types of objects mouthed, including many non-toy objects. Children mouth pacifiers significantly longer than other objects, regardless of age. Significantly increased mouthing time of all non-pacifier objects has been reported for children in the 0-18 month range compared to the 19-36 month range. Because extraction of DINP does not correlate with content, because extraction is highly variable across both laboratory procedures and human subjects, and because the number and distribution of children's and other products containing DINP is unknown, amounts of consumer-product related DINP exposure within the U.S. population cannot be well characterized. Furthermore, in the largest available studies of mouthing behavior in the youngest and potentially highest risk groups (0-36 months old), important covariates such as developmental age, physical condition, ethnicity, and other sociodemographic indicators are not reported. Therefore, estimated DINP exposures to children through toys and/or bedding/shoes/clothing, and to adults from shoes/clothing, are preliminary at best.

Table IV-7. Summary of CHAP estimates of potential DINP exposure

Age group	**Type of plastic object**	**Plausible upper-bound estimates of ingested DINP (mg $kg^{-1}d^{-1}$)**
0-18 months	Non-pacifier PVC toy	0.28
19-36 months	Non-pacifier PVC toy	0.066

Estimates obtained above by the CHAP (summarized in Table IV-7) indicate that mouthing of DINP-containing toys may result in ingestion exposures ranging from 0.07 to 0.28 mg $kg^{-1}d^{-1}$ in a reasonably highly exposed subset of children. Some amount of percutaneous DINP exposure is expected to arise from dermal or oral contact with bedding or clothing articles made of PVC materials plasticized using DINP, to the extent these materials exist. Such exposures are expected to be greatest from plastic products for which the duration of routine dermal contact is greatest. These products include plastic playpens, raincoats/ponchos, rain pants, rain shoes/boots, shoes/sandals/thongs (e.g., "jelly" sandals), rain hats, rubber (e.g., dishwashing) gloves, jewelry, and plasticized toddler training pants—again, only to the extent that any of these products contain DINP. As discussed in Appendix A, data from *in vitro* and *in vivo* studies involving dermal exposures to neat DINP are consistent with the hypothesis that all such potential dermal exposures to DINP are negligible, whereas current theoretical models predict non-negligible DINP uptake by skin or oral mucosa in exposures that involve contact with dilute aqueous DINP. In the absence of detailed data on the prevalence of DINP in consumer products that are in sustained contact with external skin and/or oral mucosa, and in view of present fundamental uncertainty concerning the magnitude of dermal DINP uptake discussed above (Section IV.C), current estimates of potential dermal exposure from such products remain speculative. In view of the range of potential dermal exposures to DINP that can be estimated for a few types of consumer products (see Appendix A), it is clear that additional experimental data could substantially reduce current uncertainties concerning the magnitude of potential dermal exposures to DINP and other phthalate esters from consumer products.

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V. METABOLISM AND PHARMACOKINETICS

A. Absorption

Oral absorption of ^{14}C -DINP was studied (Hazleton, 1972) in conditioned (pre-treatment with non-labeled DINP) and non-conditioned male albino rats. Within 72 hours, 85% of the administered dose was excreted in the feces, most within the first 24 hours. The rest was excreted in urine (average of 12%) or remained in the tissues (trace amounts). Thus, the oral absorption was approximately 12%. In studies at Midwest Research Institute (1983), male and female Fischer 344 rats were dosed orally either in a single or in 5 daily doses of 50, 150, or 500 mg kg^{-1}. At least 49% of the single low dose was absorbed. Absorption was decreased at the high single dose and at all doses following repeated exposures.

B. Biotransformation

Most of the ^{14}C collected in the urine of rats following a single oral dose of ^{14}C -DINP was in the form of phthalic acid or side-chain oxidation products of the monoester (MINP) (Midwest Research Institute, 1983). The relative amount of phthalic acid in the urine decreased at the high dose. The monoester itself, as well as the diester, was present in only trace amounts. In feces, 8 and 41% of the radioactivity was associated with the diester following administration of a low (50 mg kg^{-1}) or a high (500 mg kg^{-1}) oral dose of ^{14}C -DINP. This indicates saturation of metabolism at the high dose. The remainder of the fecal radioactivity was associated with the monoester or its side-chain oxidation products. Major metabolites in liver were the monoester and its side-chain oxidation products. The same metabolites were in testes along with phthalic acid. Fat contained the monoester and its oxidation products. Repeated exposures revealed similar metabolites in the tissues. In summary, in the rat, DINP was de-esterified to the monoester, which was further metabolized by side-chain oxidation of the ester group or by hydrolysis to phthalic acid. Formation of oxidation products appeared to increase following the high dose or repeated dosing, while the hydrolysis to phthalic acid decreased (Midwest Research Institute, 1983).

C. Distribution

In albino rats receiving 0.5 mL of ^{14}C -DINP after 5 days of dosing with the same amount of unlabeled DINP (Hazleton, 1972), after 3 days no tissue studied had over 0.001% of the administered dose. The liver contained the most radioactivity on a total tissue basis. In male and female Fischer 344 rats receiving single or repeated oral doses of ^{14}C-DINP (Midwest Research Institute, 1983), radioactivity also cleared from the tissues rapidly, but analysis of tissues within 1 hour after the exposure indicated that the highest levels were in liver (4.7% of administered dose), kidneys (0.31%), and blood (1.62 %). Fat and

testes contained small amounts of metabolites. No bioaccumulation occurred over 72 hours postdating.

D. Excretion

The major routes of excretion for orally administered DINP in rats were urine and feces, with about equal amounts excreted by either route at low doses, but more excreted in feces at high doses (Midwest Research Institute, 1983). Repeated dosing caused no accumulation of DINP or its metabolites in blood or tissue, but resulted in increased formation and elimination of the monoester side-chain oxidation products (Midwest Research Institute, 1983).

E. References

Hazelton (1972) *Metabolism study of ^{14}C phthalate ester in rats - Final Report 32563*. Vienna, VA. For Esso Research and Engineering Company.

Midwest Research Institute (1983) *Single and Repeated Oral Dose Pharmacokinetics of ^{14}C-Labeled Diisononyl Phthalate*. Kansas City, MI. For Exxon Corporation, Medical Department, Research and Environmental Health, P.O. Box 235, East Millstone, NJ.

VI. SYSTEMIC TOXICITY

This section reviews the data on the adverse systemic effects of DINP observed in laboratory studies, primarily in rodents. The toxicological information was derived from summary reports, publications in the scientific literature, and unpublished laboratory reports as indicated. Sources of primary data or the original publications were examined insofar as they were available. Otherwise, summary reports were relied on. In text and tables, statistical significance was indicated by probability level when this could be determined. The author's designation as statistically significant was accepted when the probability level was unknown.

A. Liver Effects

Hepatomegaly

In the chronic studies of DINP, the presence of hepatocellular neoplasms and the involvement of the liver in mononuclear cell leukemia affected the liver weights of treated as well as control rats and made it difficult to evaluate any effects on liver weight. The subchronic studies in mice and rats, without these complications, showed that treatment with DINP in the diet increased liver weights in less than four weeks after the start of treatment (see Table VI-1). Studies of longer duration (13 weeks) and with larger numbers of rats and mice showed that dosages of DINP above 2600 mg $kg^{-1}d^{-1}$ for mice and above 292 mg $kg^{-1}d^{-1}$ for rats increased liver weights. The no observed adverse effect level (NOAEL) for liver enlargement in mice was 904 mg $kg^{-1}d^{-1}$ and in rats was 146 mg $kg^{-1}d^{-1}$.

Liver weights were not apparently increased by DINP in two studies in limited numbers of subhuman primates (Table VI-1). In marmosets treated with DINP by gavage for 13 weeks, the mean absolute and relative liver weights of all treatment groups were greater than the liver weights of the controls, but none of the differences was statistically significant. The increases in liver weights did not show a relationship to increasing dose; in fact, in males the greatest liver weights were in the low dose group and in females in the mid-dose group. The liver weights of male and female marmosets which received 500 mg $kg^{-1}d^{-1}$ of the peroxisome proliferator clofibrate were also increased but again, the differences were not statistically significant. The small number of animals per group (4 /sex/group for DINP and 3/sex/group for clofibrate) makes it difficult to rule out a treatment effect on the liver. However, the absence of histopathological changes and the anomalous dose-response relationship makes it unlikely that the differences in liver weights are related to treatment.

Spongiosis Hepatis

Spongiosis hepatis is a lesion of the perisinusoidal cells of the liver (the Ito cells) whereas the carcinogenic and other toxic effects of DINP on the liver involved hepatocytes, which are the predominant cell type in the liver. Spongiosis hepatis, also called cystic or

microcystic degeneration, is classified as a degenerative lesion. This change has been found in livers of rodents and fish following exposure to substances (e.g., nitrosamines) that induce cancer in these species (Stroebel et al., 1995).

Liver slides from the two chronic rat studies were reviewed by the Pathology Working Group (EPL, 1999), a group of pathologists formed to review the critical pathology reported in these studies. The Pathology Working Group used diagnostic criteria developed by the National Toxicology program for liver lesions in medaka (Boorman et al., 1997). They summarized these as follows (EPL, 1999):

- Cystic spaces or large vacuoles between hepatocytes
- [Cystic spaces] incompletely or not lined by endothelium
- Spaces filled with erythrocytes, eosinophilic flocculent or fibrillar material or eosinophilic proteinaceous fluid.

Table VI-2 shows the occurrence of spongiosis hepatis at the time of terminal sacrifice (2 years) in the two chronic rat studies conducted with DINP. The incidences shown in the table were the consensus diagnoses from the slide review performed by the Pathology Working Group.

Spongiosis hepatis occurred in control males of both studies but not in any female controls of the Moore et al. (1998a) study. Among control and treated groups, the incidences of spongiosis hepatis were uniformly less in the Moore et al. (1998a) study.

This can be explained by the different sampling of the liver lobes in the two studies; 4-5 sections of livers were examined in the Lington et al. (1997) study versus 1-2 sections in the Moore (1998a) study. In the Moore (1998a) study, spongiosis hepatis was not reported in control rats that were sacrificed before the terminal sacrifices. The incidence of spongiosis hepatis in unscheduled deaths was not reported for the Lington et al. (1997) study. In the Moore (1998a) study, spongiosis hepatis in control males was moderate or minimal in severity.

Both chronic studies of DINP (Lington et al., 1997; Moore, 1998a) reported increased incidences of treated male rats with spongiosis hepatis. The Histopathology Peer Review and Pathology Working Group confirmed these findings (See Table VI-.2). The incidence of spongiosis hepatis in treated female rats was similar to controls in both studies. In both studies the incidences of treated male rats with spongiosis hepatis showed a dose-response relationship. In the Lington et al. (1997) study, 0.3% was the lowest effect level; while in the Moore (1998a) study the lowest effect level was 0.6%. The corresponding NOELs were 15 and 88 mg $kg^{-1}d^{-1}$, respectively, for the two studies. Spongiosis hepatis occurred in occasional treated rats of both studies at the time of the interim sacrifices. The earliest occurrences after treatment for 27 weeks were in a female rat of the group receiving 6000 ppm of the Lington et al. (1997) study and a male rat also receiving 6000 ppm in the Moore (1998a) study. At week 79 in the Lington et al. (1997) study, spongiosis hepatis occurred in 8 male rats; 1 rat of the 300 ppm group, 4 rats of the 3000 ppm group, and 3 rats of the 6000 ppm group.

Table VI-1. Effect of DINP on Liver Weights in Acute and Subacute Studies

Species	Study Duration	Dosage Level (mg $kg^{-1}d^{-1}$)	Increased Liver Weights	NOAEL	Remarks[a]	Reference
Mice	2 and 4 wks.	0.05 and 0.60% in feed (no data)	Yes	0.05%	5 male mice/group; Increased liver weight at 2 and 4 weeks. *P.L.*< 0.05.	Smith et al. (2000).
	13 wks.	0.15, 0.40, 1.0 and 2.0% in feed (M: 340, 904, 2365 and 5472 mg $kg^{-1}d^{-1}$. F: 389, 1041, 2834 and 6070 mg $kg^{-1}d^{-1}$)[b]	Yes	0.40%	10 mice/sex/group	Bankston (1992) See also Moore (2000)
Rats	2 and 4 wks.	0.10 and 1.2% in feed (no data)[c]	Yes	0.10%	5 male rats/ group; Increased liver weight at 4 weeks. *P.L.*< 0.05.	Smith et al. (2000) .
	3 wks.	0.06, 0.12 and 0.25% in feed (M: 639, 1192 and 2195 mg $kg^{-1}d^{-1}$. F: 607, 1198 and 2289 mg $kg^{-1}d^{-1}$) [b]	Yes	None	5 rats/sex/group	BIBRA (1985); Barber et al (1987).
	4 wks.	0.2, 0.67, and 2.0% in feed (no data) [c]	Yes	0.2 %	No. of rats/sex/group not known. Increased liver weight.	Shellenberger et al. (1983) (abstract)
	13 wks.	0.10, 0.30, 0.6, 1.0 and 2.0% in feed (50, 150, 320, 530 and 1260 mg $kg^{-1}d^{-1}$) [b]	Yes	0.10%	15 rats/sex/group. Increased liver weight in both males and females. Statistical significance not provided.	Bird et al. (1986) (abstract).
	13 wks	0.25, 0.50, 1.0 and 2.0% in feed (M: 146, 292, 584 and 1168 mg $kg^{-1}d^{-1}$. F: 182, 364, 728 and 1456 mg $kg^{-1}d^{-1}$) [b]	Yes	0.25%	10 rats/sex/group. Liver weight increased in males and females. Statistical significance not provided.	Myers (1991)

Table VI-1. Effect of DINP on Liver Weights in Acute and Subacute Studies (continued)

Species	**Study Duration**	**Dosage Level** (mg $kg^{-1}d^{-1}$)	**Increased Liver Weights**	**NOAEL**	**Remarks**[a]	**Reference**
Marmosets	13 wks	100, 500, and 2500 mg $kg^{-1}d^{-1}$ by gavage.	Not determinable	2500 mg $kg^{-1}d^{-1}$	4 marmosets/sex/group; absolute and relative liver weights of all treated groups greater than controls; no dose response and not statistically-significant	Hall et al. (1999)
Cynomolgus Monkey	14 days	500 mg $kg^{-1}d^{-1}$	No	500 mg $kg^{-1}d^{-1}$	4 monkeys per group	Pugh et al. (2000)

[a] P.L. is the probability level, indicating statistical significance of the result.
[b] Estimated Dietary Dose
[c] No data indicates that there were no data given to allow calculation of dosages.

Table VI-2. Incidence of Spongiosis Hepatis in Rats Treated with DINP, as Reported by the Pathology Working Group (EPL, 1999) -- Terminal Sacrifice

Study	Group No.	Dietary Concentration (mg $kg^{-1}d^{-1}$)	Sex	Number of Rats	Number of Rats with Spongiosis Hepatis	Incidence (%)
Lington et al. (1997)	1	0	M	81	22	27.2
			F	81	4	4.9
	2	0.03% (M: 15; F:18 mg $kg^{-1}d^{-1}$)	M	80	24	30.0
			F	81	1	1.2
	3	0.30% (M: 152; F:184 mg $kg^{-1}d^{-1}$)	M	80	51	63.8**
			F	80	3	3.8
	4	0.60% (M: 307; F: 375 mg $kg^{-1}d^{-1}$)	M	80	62	77.5**
			F	80	4	5.0
Moore (1998a)	1	0	M	55	6	10.9
			F	55	0	0
	2	0.05% (M:29;F:36 mg $kg^{-1}d^{-1}$)	M	50	6	12.0
			F	50	0	0
	3	0.15% (M: 88; F: 109 mg $kg^{-1}d^{-1}$)	M	50	3	6.0
			F	50	0	0
	4	0.60% (359;F:442 mg $kg^{-1}d^{-1}$)	M	55	18	32.7**
			F	55	1	1.8
	5	1.2% (M:733;F:885 mg $kg^{-1}d^{-1}$)	M	55	26	47.3**
			F	55	2	3.6

Table VI-2. Incidence of Spongiosis Hepatis in Rats Treated with DINP, as Reported by the Pathology Working Group (EPL, 1999) -- Terminal Sacrifice (continued)

Study	Group No.	Dietary Concentration (mg $kg^{-1}d^{-1}$)	Sex	Number of Rats	Number of Rats with Spongiosis Hepatis	Incidence (%)
Moore (1998a) (cont.)	6	1.2% Recovery (M: 637; F: 774 mg $kg^{-1}d^{-1}$)	M	50	10	20.0
			F	50	0	0

** P.L. ≤ 0.01, one-tailed Fisher's exact test (Babich and Greene, 2000)

Summary histopathological data in the Moore (1998a) study reported the severity of spongiosis hepatis in both male and female rats at terminal sacrifice**.** The most severe lesion was seen in a male rat of the highest dose group. Otherwise, the average grades for the treated groups showed no apparent increase in severity with increasing dose. Severity grades for the male rats only are shown in Table VI-3 since the incidence in female rats was so few.

Table VI – 3. Severity of Spongiosis Hepatis in Male Rats of Moore (1998a) Study at Time of Sacrifice

Group	No. of Male Rats	No. of Male Rats with Spongiosis Hepatis	Grade			Average Grade[a]
			Grade 1	Grade 2	Grade 5	
1	41	5	1	4		1.8
2	34	4	4			1.0
3	39	2		2		2.0
4	36	12	3	9		1.8
5	32	18	5	12	1	1.9
6	29	7	3	4		1.6

[a] For rats with spontaneous hepatis, average grade = (No. of rats with grade 1 × 1) + (No. of rats with grade 2 × 2) divided by number of rats with lesion. For Group 1: (1 × 1)+ (2 × 4) = 9 ÷ 5 = 1.8.

The relationship of spongiosis hepatis to peroxisome proliferation in the livers of rats exposed to DINP is not clear. While DINP induced peroxisome proliferation in both sexes of rats and mice, spongiosis hepatis was increased in only the male rats. Moreover, spongiosis hepatis occurred in control rats and in treated rats at dosages which did not apparently induce peroxisome proliferation (Bird et al., 1987). All of this would suggest that spongiosis hepatis is unrelated to peroxisome proliferation. Similarly, spongiosis

hepatis was not apparently related to the occurrence of mononuclear cell leukemia, despite the liver involvement in this disease. The PWG (EPL, 1999) examined the latter relationship by comparing the incidence of rats with both lesions to the incidence of spongiosis hepatis without mononuclear cell leukemia. Of those animals that had spongiosis hepatis, about half also had mononuclear cell leukemia.

While the occurrence of increased incidence in treated male rats with spongiosis hepatis was clearly related to treatment with DINP in a dose-related fashion in both studies, the relationship of spongiosis hepatis to carcinogenesis in the liver is less clear. Some neoplastic livers also showed spongiosis hepatis and in some livers, areas of spongiosis hepatis were observed as part of the neoplasm. However, there is no evidence that spongiosis hepatis is part of a neoplastic process. In the recovery group of the Moore (1998a) study, spongiosis hepatis occurred at a lower incidence three months after treatment was discontinued than in the group receiving the same dose level continuously. The occurrence of spongiosis hepatis in the recovery group and no increased incidence of neoplasms would indicate that spongiosis hepatis is not a progressive lesion. The lack of a dose-related increase in severity supports this interpretation. On the other hand, studies of spongiosis hepatis in livers of rat treated with N-nitrosomorpholine have shown an increased incorporation of ^{3}H-thymidine that was maintained months after the withdrawal of carcinogen (Stroebel et al. 1995), which would presumably be the hallmark of a progressive lesion.

Thus, in rats treated with DINP, the following observations can be made about the occurrence of spongiosis hepatis:

- Spongiosis hepatis occurred in rats, but not in mice.
- Spongiosis hepatis occurred with higher incidences in control male rats than in female rats.
- Male rats but not female rats in two chronic studies showed a dose-related increased incidence of spongiosis hepatis.
- In male treated rats there was no indication of increasing severity with increasing dosage of DINP.
- Spongiosis hepatis was found earlier in treated rats than in controls in the preterminal sacrifices, but there was no obvious relation to dosage.
- In a recovery group, the incidence of rats with spongiosis hepatis was lower than in a group which received the same dosage continuously, but still about twice the incidence in the control group.
- There was no apparent relationship between spongiosis hepatis and peroxisome proliferation or the occurrence of mononuclear cell leukemia.
- Similarly, there was no apparent relationship between the occurrence of spongiosis hepatis and the occurrence of hepatocellular neoplasms.

B. Kidney Effects

In rats, increased relative weights of the kidneys were seen after treatment with DINP for 21 days as well as in both long term studies (Table VI-4). In the Lington et al. (1997)

study, dietary levels of 0.3 and 0.6% increased relative weights of kidney of both male and female rats. In the Moore (1998a) study, increased relative kidney weights occurred in groups receiving 0.6% and greater at week 79 and at the two year terminal sacrifices. In this study, increased mineralization of renal papillae was reported in male rats in the same groups manifesting increased kidney weights. However, it is unlikely that the histological effects reported (mineralization of renal papillae in male rats and pigmentation of kidney tubule cells) accounts for the increased weights of the kidneys.

Table VI-4. Effect of DINP on Kidneys of Mice and Rats

Species	**Study Duration**	**Dose Groups ($mg\ kg^{-1}d^{-1}$)**	**Kidney Changes**	**NOAEL**	**Reference**
Mouse	13 wks.	0.15, 0.40, 1.0 and 2.0% (M: 340, 904, 2365, and 5472 mg $kg^{-1}d^{-1}$. F: 389, 1041, 2834 and 6070 mg $kg^{-1}d^{-1}$)	Decreased absolute and relative kidney weights in male mice; increased relative kidney weights in female mice at highest dose.	0.15%	Bankston (1992)
	2 yrs.	0.05, 0.15, 0.40 and 0.80% (M: 90, 276, 742 and 1560 mg $kg^{-1}d^{-1}$. F: 112, 336, 910 and 1888 mg $kg^{-1}d^{-1}$)	Decreased relative kidney weights in male mice	0.15%	Moore (1998b)
			Increased nephropathy in female mice	0.4%	Moore (1998b)
Rat	3 wks.	0.06, 0.12, and 0.25% (M: 639, 1192 and 2195 mg $kg^{-1}d^{-1}$. F: 607, 1198 and 2289 mg $kg^{-1}d^{-1}$)	Increased relative kidney weight	None	BIBRA (1985)
	13 wks.	0.10,0.30, 0.60, 1.0 and 1.2% (50, 150, 320, 530 and 1260 mg $kg^{-1}d^{-1}$)	Increased absolute and relative kidney weights. Dose-related increased incidence of nephrosis.	0.10	Bird et al. (1986)
	13 wks.	0.25, 0.50, 1.0 and 2.0% (M: 146, 292, 584 and 1168 mg $kg^{-1}d^{-1}$. F: 182, 364, 728, and 1456 mg $kg^{-1}d^{-1}$)	Increased kidney weight and bun in high dose male and female rats. Increased regenerative basophilic renal tubule cells in males of 0.50% and higher.	1.0%	Myers et al. (1991)
	2 yrs.	0.03, 0.3, 0.6% (M:15, 152 and 307 mg/kg/day. F: 18, 184, and 375 mg $kg^{-1}d^{-1}$)	Increased relative kidney weights in both males and females. Increased urine volumes in high dose male rats.	0.03%	Lington et al. (1997)
	2 yrs.	0.05, 0.15, 0.60, and 1.2% (29, 88, 359 and 733 mg $kg^{-1}d^{-1}$. F: 36, 109, 442 and 885 mg $kg^{-1}d^{-1}$)	Increased relative kidney weights in male and female rats of 0.60% and 1.2% groups. Increasd urine volume in high dose males.	0.15%	Moore (1998a)
			Increased mineralization of renal papillae in male rats at 0.60 and 1.2%.	0.15%	Moore (1998a)

In male mice fed diets containing 0.4 and 0.8% DINP, relative kidney weights were reduced at the two-year sacrifices (Moore, 1998b). In this study, an increased incidence and severity of chronic progressive nephropathy occurred in female mice fed diets containing 0.8% DINP but no effect on kidney weights was reported. In the recovery group, the incidence and severity of this lesion was comparable to the control group suggesting that the exacerbation of nephropathy in female mice did not occur after treatment was discontinued.

Kidney changes in female mice and male rats did not appear to be indicative of kidneys as a target organ for carcinogenesis despite the occurrence of a small number of renal neoplasms in male rats of the Moore (1998a) study. Caldwell et al. (1999) reported an accumulation of α2μ-globulin in kidneys of high dose male rats from the Lington et al. (1997) study. The relation of α2μ-globulin nephropathy to the kidney neoplasia is discussed in another section of this report.

The NOAEL for kidney changes is 88 mg $kg^{-1}d^{-1}$ based on increased kidney weights and mineralization in the male rats of the two year study (Moore, 1998a).

C. Other Effects

Hematology

Decreased numbers of red blood cells, reduced hemoglobin concentrations, and decreased hematocrits were seen in rats fed diets containing 0.6% DINP and higher in both long term studies. The other species did not show these signs of anemia nor was anemia observed in the short term studies in rats, even at higher doses.

Clinical Chemistry

Urinalysis. In both rat and mouse long-term studies, treatment with DINP in the diet at concentrations of 0.6% and higher was associated with increased urine output and a corresponding decrease in electrolyte concentrations. These changes occurred at the same dosages that produced changes in kidney weights.

Serum Chemistry. Male rats, treated for 21 days with DINP in the diet showed decreased cholesterol and triglycerides in all treated groups (BIBRA, 1985). In the females of this study, these blood lipids were increased at the mid- and high-dose levels (1.2 and 2.5% in the diet). The serum concentrations of liver enzymes, alanine aminotransferase and aspartate aminotransferase, showed consistent increases at the mid- and high-doses in the male rats of the Lington et al. (1997) study. On the other hand, changes in these enzymes were sporadic in the Moore (1998a) study and not apparently dose-related.

Other Biochemical Changes. Total liver proteins were increased in all treated groups of rats receiving DINP in the diet for 21 days in the BIBRA study (1985). In this same

study, 11-hydroxylase and 12-hydroxylase levels were increased in males of all treated groups and in the females of the high dose group.

D. Summary of Systemic Toxicity

Table VI-5 contains a summary of the non-carcinogenic systemic toxic effects induced in laboratory animals following oral exposure to this substance. The tabled values for each parameter are the results in the most sensitive species and sex in each case. Due to the lower DINP intake of male rats as compared with female rats, male rat data were used to estimate NOAEL values. As shown, the non-carcinogenic systemic toxic effects associated with treatment with DINP began to occur at dosages of 150 mg $kg^{-1}d^{-1}$ or above.

Table VI-5. Summary of Non-Carcinogenic Systemic Toxic Effects of DINP in Most Sensitive Species and Sex

Effect	Species (Sex)	LOAEL (mg $kg^{-1}d^{-1}$)	NOAEL (mg $kg^{-1}d^{-1}$)	References
Hepatomegaly	Rat (male)	292	146	Myers (1991)
Spongiosis hepatis	Rat (male)	584 and 152	15 and 88	Lington et al. (1997) Moore (1998a)
Kidney weight increase	Rat (male)	152 and 359	15 and 88	Lington et al. (1997), Moore (1998a)
Hematological Changes	Rat (male)	307 and 359	152 and 88	Lington et al. (1997), Moore (1998b)
Urinalysis	Rat (male)	307 and 733	152 and 359	Lington et al. (1997), Moore, 1998b
Serum Chemistry	Rat (male)	152	15	Lington et al. (1997)

E. Bio/dynamics Study of Santicizer 900 in Sprague-Dawley Rats

A study of subchronic and chronic toxicity (Bio/dynamics, 1986) was also performed using Sprague-Dawley rats exposed to various concentrations of a diisononyl phthalate, Saniticizer 900 (CAS# 71549-78-5), that is chemically similar to the DINP used in the previously discussed studies. As mentioned in Section III, one form of DINP currently manufactured (CAS# 28553-12-0) is produced by converting *n*-butene (either alone, or together with isobutene) primarily to mixtures of alcohols (e.g., methyloctanols, dimethylheptanols, and/or methylethyl hexanols). The CMA (1999) has stated that "although DINP is a complex substance, it is not variable due to the stability of the alcohol manufacturing process. The two types of [DINP produced starting with *n*-butene, or with *n*-butene + isobutene] are considered commercially interchangeable." Although Santicizer 900 (CAS# 71549-78-5) was never commercialized, samples were analyzed in Germany (BASF AG). According to Mr. Patrick Harmon of BASF, "Santicizer 900 is chemically similar to the current BASF product Palatinol(R) N and to other DINPs such as CAS# 28553-12-0 that are produced from isononanol made via the dimerization of butene" (Harmon, personal communication to the CPSC, 2000).

The rats treated with Santicizer 9000 showed much the same liver changes seen in F344 rats treated with DINP except for the occurrence of focal hepatocellular necrosis. This change occurred in control rats as well as treated rats, but the incidences in treated groups of male rats were generally greater than in controls (see Table VI-6.) Among the treated male rats, no increased incidence was obvious either in the interim-sacrificed animals or in the unscheduled deaths. Furthermore, female rats at term showed no increase in incidence of focal hepatocellular necrosis. Among male rats, sacrificed at term, the incidences of rats with this change were increased in the high dose and in the low dose rats, but not in the mid-dose animals.

On the surface, these data could be used to determine safe levels for exposure to DINP. If treatment-related, the finding of foci of hepatocellular necrosis could represent a serious toxic effect. The increased incidence of rats with this change was statistically significant in male rats at dosages of 27 and 553 mg $kg^{-1}d^{-1}$ (see Table VI-6) which would result in a NOAEL lower than the 15 mg $kg^{-1}d^{-1}$ observed in the Lington et al. (1997) study discussed above for spongiosis hepatis.

Several reasons against using this lesion for determining acceptable exposure to DINP are set forth below:

1. Increased incidences of focal hepatocellular necrosis were not seen in female rats at any dosage level despite higher dosages to females.
2. Focal hepatocellular necrosis was not reported in any other studies with other rat strains treated with DINP.
3. Focal hepatocellular necrosis has not been described in acute or chronic rat studies with other phthalates.
4. No increased incidences of rats with this change occurred in the interim sacrificed rats or rats dying before scheduled sacrifices.
5. The questionable nature of the change described as “small foci of hepatocellular necrosis” as well as its high variability in female rats and in the unscheduled deaths, treated as well as controls.
6. The male treated rats do not show a consistent increased incidence with increasing dose. (The incidence in the middle dose males was less than in the low dose group.)
7. The severity was not apparently increased with increasing dosage; the lesion was described as “minimal to mild” with no observations described as “severe.”
8. The presence of treatment-related necrosis in treated animals at two years but not at one year or in spontaneous deaths is an unusual occurrence, especially when escalating dosages are used.
9. The relationship of the test article used in the Bio/dynamics study to the products children are exposed to as well as the test articles used in other toxicity studies is questionable.

Table VI-6. Incidence of male rats with focal hepatocellular necrosis (FHN) in Santicizer 900 Study in Sprague-Dawley Rats

	Group	Dosage	No.of Rats Examined	No. of Rats with FHN	Incidence
ALL RATS	I	Controls	70	5[a]	7%
	II	0.05% (27 mg $kg^{-1}d^{-1}$)	69	17[a,b]	25%
	III	0.5% (271 mg $kg^{-1}d^{-1}$)	69	11[a]	16%
	IV	1.0% (553 mg $kg^{-1}d^{-1}$)	70	23[a,c]	33%
TERMINAL RATS	I	Controls	29	0	0
	II	0.05% (27 mg $kg^{-1}d^{-1}$)	26	4	15%
	III	0.5% (271 mg $kg^{-1}d^{-1}$)	24	3	9%
	IV	1.0% (553 mg $kg^{-1}d^{-1}$)	34	15[d]	44%

[a] χ^2 =14.4, p = 0.00036, Bartholomew's test for trend.
[b] p = 0.0084, 2-tail Fisher Exact test.
[c] p = 0.00023, 2-tail Fisher Exact test.
[d] p = 0.000030, 2-tail Fisher Exact test.

Moreover, there are questions about the exact nature of the change, not only its severity in relation to treatment but also its relationship to the occurrence of other liver lesions such as liver neoplasms. The presence of a neoplasm in the liver most likely has the potential to compromise circulation in this organ and could explain the higher incidences in the terminal animals and in high dose animals.

For these several reasons, the findings of the Bio/dynamics study of Santicizer 900 in Sprague-Dawley rats are not used for calculating acceptable exposures to DINP.

F. Conclusions

The NOAEL for systemic toxic effects induced in laboratory animals by exposure to DINP is 15 mg $kg^{-1}d^{-1}$ based on the results of the Lington et al. (1997) study or 88 mg $kg^{-1}d^{-1}$ based on the results of the Moore (1998a) study.

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VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Because phthalates are known to produce reproductive and developmental toxicity, these issues have been investigated with DINP. A major, up-to-date resource for these endpoints is the "CERHR Draft Evaluation of Di-Isononyl Phthalate" (last update 8/31/00) prepared by the Center for the Evaluation of Risks to Human Reproduction (NTP/CERHR). The relevant sections of the CERHR document are reproduced with minor changes below.

A. Developmental Toxicity

There were no human studies located.

Two published prenatal developmental toxicity studies in rats were available for DINP (Waterman et al., 1999; Hellwig, et al., 1997). The protocols for the two studies were similar and included dosing of dams by gavage on gestational day (gd) 6–15 with sacrifice and evaluation of fetuses on gd 20–21. The group sizes differed. Developmental toxicity was also noted in both a one-generation and a two-generation toxicity study. The effects on pup body weight are discussed below and summarized in Table VII-1; the reproductive effects are described in Section VII-B.

Hellwig et al. (1997) performed their studies in Wistar rats (10/group) at doses of 0, 40, 200, and 1,000 mg $kg^{-1}d^{-1}$. Although sample size (n=10) was small, the aggregate of their work can logically be considered to be three separate studies of DINP. There was a degree of consistency across all studies. Effects were only observed at the highest dose. Relative kidney and liver weights were slightly increased in dams of the highest dose group (5–13%), but statistical significance was erratic. Fetal viability and body weight were unaffected in all three studies. Skeletal variations (rudimentary cervical ribs, accessory 14^{th} ribs) were numerically increased with each DINP with the number of affected fetuses per litter significantly higher than controls in two instances. There was a tendency to see dilated renal pelves at the highest dose; in one study agenesis of kidneys and ureters was assumed by the authors to be DINP-related. Skeletal (shortened and bent humerus and femur) malformations were also observed in the high-dose group of this study. It is clear that organ effects are associated with kidney and the skeletal system. For maternal and developmental effects, a NOAEL of 200 and a LOAEL of 1,000 mg $kg^{-1}d^{-1}$ were identified by the CERHR Panel for each DINP and are in concordance with effect levels identified by Hellwig et al. (1997).

The prenatal toxicity study of Waterman et al. (1999) was more informative than the Hellwig study from the standpoint of number of rats per test group and completeness of data reported. Waterman et al. (1999) tested DINP-1 in Sprague-Dawley rats (25/group) at doses of 0, 100, 500, or 1,000 mg $kg^{-1}d^{-1}$. Maternal toxicity at the highest dose consisted of decreased food intake and weight gain. The authors presented and analyzed effects on offspring as percent affected fetuses and percent affected litters. Waterman et

al. (1999) interpreted their results as indicating a LOAEL for maternal and developmental toxicity at 1,000 mg $kg^{-1}d^{-1}$ and a NOAEL of 500 mg $kg^{-1}d^{-1}$. The CERHR Panel concurred with the maternal NOAEL, but concluded there was developmental toxicity at the 500 mg $kg^{-1}d^{-1}$ dose. The CERHR Panel advised the study sponsor that there were more recent and improved methods for the statistical analysis of fetal incidence data. The sponsor performed appropriate reanalyses that the CERHR Panel reviewed and found to be consistent with the CERHR Panel interpretation of skeletal variations. The CERHR Panel concludes there is a NOAEL in the study at 100 mg $kg^{-1}d^{-1}$. The BMD estimated at a 5% excess risk level was 193 mg $kg^{-1}d^{-1}$ (95% LCL= mg $kg^{-1}d^{-1}$) for rudimentary lumbar ribs, as provided by the study sponsor (McKee, R. Personal Communication).

The CERHR Panel noted that developmental toxicity was observed in the prenatal rat studies by Waterman and Hellwig. In the study by Waterman (n=25), the urinary system was a target of effect as noted by a modest increase in dilated renal pelves at the 1,000 mg kg^{-1} dose. While only a mild increase in dilated renal pelves was observed in the three Hellwig et al. studies, in one instance more severe renal effects (hydroureter, agenesis) were seen. In studies by Waterman et al. (1999) and Hellwig et al. (1997), the skeletal system was the target of effect as observed by an increased incidence of cervical ribs and accessory 14^{th} (lumbar) ribs. These studies also evaluated the closely related phthalate DIDP where the same target organs were identified. An increase in cervical ribs and lumbar ribs was observed at the common dose of 1,000 mg $kg^{-1}d^{-1}$ in the two studies. While the effect on lumbar ribs was more pronounced, the effect on cervical ribs is of greater toxicological concern. Cervical ribs are seen infrequently in controls, but more importantly, their presence may indicate a disruption of gene expression. In addition, some scientists express concern that cervical ribs may interfere with normal nerve function and blood flow.

Differences in NOAELs between the Waterman et al. (1999) and Hellwig et al. (1997) studies, 100 and 200 mg $kg^{-1}d^{-1}$ respectively, may be due to rat strain, and certainly to dose selection.

The two-generation reproductive study by Waterman et al. (2000) suggests an adverse effect on weight gain in pups during the perinatal and pre-weaning period of life. Developmental landmarks of reproductive tract development, identified as a sensitive target with other phthalates, were not examined. F_1 mean pup body weight was significantly reduced on prenatal day (pnd) 0 in males at 0.8% DINP (555 and 1026 mg kg^{-1} during gestation and lactation, respectively, as calculated by study sponsors). On pnd 7 and 14, mean male and female pup body weights were significantly reduced at 0.4% (287 and 539 mg $kg^{-1}d^{-1}$ during gestation and lactation, respectively) and 0.8% and by pnd 21, mean male and female body weights were reduced at all dose levels. In the F_2 generation, mean female pup body weights were significantly reduced at 0.4 and 0.8% on pnd 4, 7, 14, and 21 and at 0.2% (143 and 285 mg $kg^{-1}d^{-1}$ during gestation and lactation, respectively) at pnd 7. Mean male pup body weights were significantly reduced at 0.4 and 0.8% at pnd 7, 14, and 21. The LOAEL for developmental effects was therefore identified as 0.2% (143–285 mg $kg^{-1}d^{-1}$ during gestation through lactation) by the CERHR Panel.

Studies with 2 isononyl alcohols, differing in degree of branching, demonstrated clinical signs and symptoms in pregnant rats at doses of 720 mg $kg^{-1}d^{-1}$ and higher (Hellwig and Jackh). Table and text discrepancies in dose values and reported effects at the higher dose levels were noted. Toxicity was more severe with type 1 isononyl alcohol, the alcohol that had a higher degree of branching. Maternal mortality was seen at the highest dose (1,440 mg $kg^{-1}d^{-1}$) with both alcohols and in the type 1 alcohol at 1,080 mg $kg^{-1}d^{-1}$. Fetal malformations and/or variations occurred at 1,440 mg $kg^{-1}d^{-1}$ and at 1,080 mg $kg^{-1}d^{-1}$. Slight effects that may be associated with treatment were observed at 720 mg $kg^{-1}d^{-1}$. A dose of 144 mg $kg^{-1}d^{-1}$ was without effect for both isonoyl alcohols.

Summary of Developmental Toxicity

There are adequate data available in rats to determine that prenatal oral exposure to DINP-1 results in developmental toxicity. The results of the Waterman et al. (1999) and the Hellwig et al. (1997) studies were remarkably consistent with respect to DINP-1. In both studies, exposure to DINP-1 resulted in increases in lumbar and cervical ribs. In addition, the effective dose levels were similar. Hellwig et al. identified a LOAEL of 1,000 mg $kg^{-1}d^{-1}$ and a NOAEL of 200 mg $kg^{-1}d^{-1}$ with a sample size of 10/group. The CERHR Panel identified an effect level of 500 mg $kg^{-1}d^{-1}$ from the Waterman et al. (1999) study (sample size of 25/group) and 100mg $kg^{-1}d^{-1}$ level represented a NOAEL. In addition, Hellwig et al. (1997) showed some similarities among the three DINPs in that each resulted in an increase in lumbar and cervical ribs. It is clear that the urinary and skeletal systems are target organs where developmental toxicity is observed. The data from the two-generation dietary study are sufficient to demonstrate an effect on postnatal growth, with a LOAEL of 143–285 mg $kg^{-1}d^{-1}$ and no NOAEL. The reduced growth is consistent in both studies. Neither prenatal study extended dosing into the late gestation period that has been shown to be a critical window of development for other phthalates. In addition, the study designs did not allow for assessment of postnatal sexual maturation. The issue of late gestational exposure was addressed in a two-generation reproductive toxicity study reviewed in Section VII-B. Confidence in the isononyl alcohol study is limited due to table and text discrepancies in dose values and reported effects at the higher dose levels. The study is adequate to ascribe maternal and developmental toxicity at these higher doses and to assume the lowest dose was without effect.

B. Reproductive Toxicity

Structural and functional reproductive effects were examined in one- and two-generation feeding studies in rats that included *in utero* exposure during the entire duration of pregnancy (Waterman et al., 2000). In the one-generation dose range finding study, rats were administered dietary levels of 0, 0.5, 1.0, or 1.5% DINP and in the two-generation study, rats were administered dietary levels of 0, 0.2, 0.4, or 0.8% DINP. In the two-generation study, reproductive parameters including mating, fertility, and testicular histology were unaffected in both generations at the highest dose (0.8%; 665–779 and 696–802 mg $kg^{-1}d^{-1}$ in males and females, respectively) and this dose was identified as the reproductive NOAEL. Developmental effects were observed, including decreased

pup weight gain (most marked on pnd 21). The effects on pup weight gain are discussed in greater detail under Section VII-A. Histologic effects included mild hepatic eosinophilia in both sexes of parental rats in all dose groups of both generations and dilated renal pelves in F_1 parental males of the mid- and high-dose groups. The results of the study are consistent with the one-generation pilot study that was previously conducted. In the one-generation study, fertility was unaffected in male and female rats exposed to dietary DINP concentrations as high as 1.5% (966–1,676 and 1,114–1,694 mg $kg^{-1}d^{-1}$ in males and females, respectively). The findings of these studies indicate that male and female rat fertility and structure of reproductive organs are unaffected by exposure to DINP at a maternal dose of 555–1,129 mg $kg^{-1}d^{-1}$ during gestation and lactation, respectively, and adult exposure to concentrations as high as 1,676 mg $kg^{-1}d^{-1}$ in males and 1,694 mg $kg^{-1}d^{-1}$ in females.

Sensitive reproductive endpoints have been examined in a single dose (750 mg $kg^{-1}d^{-1}$) study of DINP-1 orally administered to rat dams from gestational day 14 to postnatal day 3 (Gray et al., 2000). This DINP dose reduced pregnancy weight gain by 14 gm. The DINP-treated male pups displayed a significantly increased incidence of female-like areolas/nipples (22.4% versus 0% in the controls; litter mean analysis, $p<0.01$). In addition, DINP induced a significant level of reproductive malformations on an individual animal basis (7.7% versus 0% for controls; $p<0.05$). The reproductive malformations were consistent with an antiandrogenic effect during development, and included permanent nipples, small and atrophic testes, fluid-filled testes, and agenesis of the epididymis. DINP-induced reproductive malformations were much less frequent than those observed following exposure of dams to 750 mg $kg^{-1}d^{-1}$ diethylhexylphthalate or butylbenzylphthalate (Gray et al., 2000).

Mode of Action

Steroid/Hormone Activity. DINP exhibited no activity in an *in vitro* assay that measured binding of phthalates to estrogen receptors (Zacharewski, et al., 1998) and in an assay of estrogen-induced gene expression (Harris et al., 1997). The assays did not include the addition of esterases or lipases to metabolize the DINP to MINP. *In vivo* assays demonstrated that DINP does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats. Thyroid and estrogen serum levels were unaffected in adult marmosets at doses as high as 2,500 mg $kg^{-1}d^{-1}$ for 13 weeks (Hall et al., 1999). The antiandrogenic effects of perinatally administered phthalates are apparently the result of lowered testosterone levels during development rather than a direct effect of the phthalate on the androgen receptor (Parks et al., 2000).

Table VII-1: Summary of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies

Protocol & Study	**NOAEL** **(mg $kg^{-1}d^{-1}$)** **[Benchmark dose – ED_{05} in mg $kg^{-1}d^{-1}$]**	**LOAEL (mg $kg^{-1}d^{-1}$) and Effects**		**Developmental Effects Observed at Higher Dose Levels**
		Maternal	Developmental	Developmental
Prenatal gavage study in Wistar rats. 10/group/study received 0, 40, 200, or 1,000 mg $kg^{-1}d^{-1}$ on gd 6–15. Dam and pups examined in late gestation. DINP-1, DINP-2, and DINP-3 (Hellwig et a., 1997a)	200 Maternal & Developmental	1,000 ↑Kidney and liver weights.	1,000 ↑Cervical and lumbar ribs – all. ↑Urogenital and skeletal malformation with 1 DIDP.	N/A
Prenatal gavage study in Sprague-Dawley rats. 25 per group received 0, 100, 500, or 1,000 mg $kg^{-1}d^{-1}$ on gd 6–15. Dams & pups examined in late gestation. DINP-1 (Waterman et al., 1999)	500 (Maternal) 100 [a] (Developmental). [MLE (95%LCL): 193 (162) for lumbar ribs]	1,000 ↓Weight gain.	500 ↑ Fetuses with vertebral variations.	↑ Fetuses and litters with visceral variations (mainly dilated renal pelves). ↑ Fetuses and litters with lumbar ribs. ↑ Fetuses with cervical ribs.
Two generation reproductive dietary study in Sprague-Dawley rats. 30 per group were fed diets with 0, 0.2, 0.4, or 0.8% from 10 weeks prior to mating (0, 182─197, 356─397, and 696─802 mg $kg^{-1}d^{-1}$) through gestation (0, 143─146, 287─288, and 555─560 mg $kg^{-1}d^{-1}$ and lactation 0, 254─285, 539─553, and 1,026─1,129 mg $kg^{-1}d^{-1}$ during lactation)[b]. DINP-1 (Waterman et al., 2000)[c]	None [250 (95% LCL) for decreased pup weight gain]	143-285 ↑ Mild histological liver changes in F_0 and F_1. ↑Kidney weight in F_0.	143-285 ↓ Weight gain on pnd 21in F_1. ↓ Weight gain on pnd 7 in F_2 females.	↓ Weight gain on pnd 0 (males), 7, 14, and 21 in F_1. ↓ Weight gain on pnd 4 (female), 7, 14, and 21 in F_2.

[a] NOAEL selected by CERHR Panel is lower than study author's selection.
[b] Range of doses for F_1 and F_2 dams.
[c] Only maternal and developmental effects were listed in this table. Reproductive and male systemic effects are listed in Table VII-2.

Summary of Reproductive Toxicity

The studies did demonstrate consistent effects on the liver (weight and histology) and kidney (weight). In the standard studies which assessed the usual endpoints of reproductive function, such as fertility, the data demonstrate no likely reproductive toxicity at doses up to 779(M)–802(F) mg $kg^{-1}d^{-1}$ in the two-generation study or at 1,676(M) –1,694(F) mg $kg^{-1}d^{-1}$ in the one-generation study. However, a recent study which examined sensitive endpoints indicated that DINP oral exposures of rat dams at 750 mg $kg^{-1}d^{-1}$ during critical stages of male reproductive tract development (gestational day 14 to postnatal day 3) produced a significant increase in reproductive tract malformations.

C. Conclusions

No human data indicative of reproductive or developmental toxicity associated with DINP exposure have been identified; therefore, the evaluation of these endpoints relies upon animal studies.

There is adequate data to determine that prenatal oral exposure of rats to DINP results in developmental toxicity with a NOAEL of 100 - 200 mg $kg^{-1}d^{-1}$. A consistent finding among the two prenatal developmental studies was the occurrence of kidney and skeletal system abnormalities. A two-generation study in the rat suggested an adverse effect upon pup weight gain with a LOAEL of 250 mg $kg^{-1}d^{-1}$. Male reproductive tract malformations consistent with an antiandrogenic effect are observed following high dose exposure of rat dams during critical stages of fetal male reproductive tract development.

Studies in rats at a high dose indicate an adverse effect on pup weight gain and male reproductive tract malformations consistent with an antiandrogenic effect. However, the CHAP concludes that there is no evidence that humans receive DINP doses from DINP-containing consumer products that are plausibly associated with an increased risk of reproductive and developmental processes in humans due to DINP exposure.

Table VII-2: Summary of NOAELs and LOAELs and Major Effects in Reproductive Toxicity Studies

Protocol & Study	NOAEL ($mg\ kg^{-1}d^{-1}$)	LOAEL ($mg\ kg^{-1}d^{-1}$) and Effects		Reproductive Effects Observed at Higher Dose Levels
		Repro	Systemic	
Two-generation reproductive dietary study in Sprague-Dawley rats. 30 per group were fed diets with 0, 0.2, 0.4, or 0.8% (Males: 0, 165–189, 331–379, and 665–779 $mg\ kg^{-1}d^{-1}$, Females: 0, 182–197, 356–397, and 696–802 $mg\ kg^{-1}d^{-1}$ [a]) from 10 weeks prior to mating through gestation and lactation. DINP-1 (Waterman et al., 2000)[b]*	665–779 (M); 696–802 (F) (Reproductive) none (Systemic)	No effects on reproductive structure or function.	M: 165–189; F: 182–197 ↑ Mild liver effects in F_0 and F_1. ↑Kidney weight In F_0 females.	None

[a] Doses during the premating period-Combined for F_0 and F_1 rats.
[b] Only effects in parental rats are listed. Developmental effects are listed in Table VII-1.

D. References

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VIII. GENOTOXICITY

A. Introduction

DINP is part of a diverse group of chemicals collectively referred to as peroxisome proliferators. Peroxisome proliferators are classic non-genotoxic carcinogens since they appear to result in liver cancer without causing genotoxic damage. In this section, the evidence examining the genotoxicity of DINP will be evaluated.

B. Mechanism

The mechanisms by which peroxisome proliferators cause hepatocarcinogenesis and tissue toxicity are not well characterized. One hypothesis is that peroxisome proliferation causes gene mutations by oxidative damage resulting from increased intracellular concentration of H_2O_2 produced by peroxisomal acyl CoA oxidase, which in the presence of metals could facilitate free radical formation (Reddy et al., 1989). This is thought to occur as a result of the large induction of acyl CoA oxidase caused by peroxisome proliferators (Nemali et al., 1988; Marsman et al., 1988), in the absence of significant upregulation of catalase (Nemali et al., 1988; Conway et al., 1989). Evidence demonstrating intracellular oxidative damage resulting from peroxisome proliferators is inconsistent. Peroxide-modified lipids have been detected in hepatocytes from rats treated with either Wy-14,643, clofibrate, or DEHP (Conway et al., 1989; Marsman et al., 1992). However, more sensitive measures of oxidative damage including ethane exhalation and hepatic levels of esterified F2-isoprostanes are not markedly influenced by administration of the potent peroxisome proliferator Wy-14,643 (Conway and Popp, 1995; Soliman et al., 1997). Evidence showing oxidative damage to DNA resulting from peroxisome proliferators is also inconsistent. DNA damage in the form of 8-hydroxydeoxyguanosine (8-OH-dG) has been reported in liver from rats after long-term treatment with peroxisome proliferators including DEHP (Kasai et al., 1989; Takagi et al., 1990), although the increase in 8-OH-dG residues are small (<2-fold), is not sustained with chronic DEHP treatment (Takagi et al., 1990; Cattley and Glover, 1993), and the correlation between 8-OH-dG levels and tumor multiplicity is weak (Marsman et al., 1988; Cattley and Glover, 1993). One study suggested that the increase in 8-OH-dG levels resulting from peroxisome proliferators is due, in part, to increased 8-OH-dG of mitochondrial DNA (Sausen et al., 1995), although this study may be flawed due to the absence of antioxidants in the preparations (Doull et al., 1999). In contrast to the studies demonstrating oxidative damage to DNA, no measurable increase in 8-OH-dG or thymidine glycol was reported in rat liver after administration of nafenopin (Hegi et al., 1990). Interestingly, there is also evidence that DNA repair pathways may be induced as a result of treatment with peroxisome proliferators. Expression of N-methyl purine-DNA glycosylase (MPG) and urinary excretion of ethenoadenine (a DNA adduct formed after lipid peroxidation and repair by MPG) are increased after subchronic exposure to Wy-14,643 (Rusyn et al., 1999). This suggests that DNA repair enzymes may correct some, if not all, of the putative oxidative damage resulting from peroxisome proliferators. This idea has not been critically evaluated to date.

C. Genotoxicity Assays

Despite evidence that peroxisome proliferators cause intracellular oxidative damage to lipids or DNA, these chemical have consistently been shown to be non-genotoxic carcinogens (Doull et al., 1999; Ashby et al., 1994; Galloway et al., 2000; Lefevre et al., 1994; Gonzalez et al., 1998). Numerous assays designed to determine the genotoxicity of chemicals have been utilized to assess the effect of peroxisome proliferators including mutation assays, genotoxicity assays, DNA damage assays, chromosomal damage assays and cell transformations. DINP has been tested in the Ames assay, Chinese hamster ovary cells for chromosomal alterations, the mouse lymphoma forward mutation assay (L5178Y TK +/- cell line), the primary rat hepatocyte unscheduled DNA synthesis assay, and an *in vitro* transformation assay using Balb/c-3T3 A31 mouse cells (Table VIII-1) Recent evidence suggests that chromosomal aberrations reported for Wy-14,643 or MEHP are secondary to cytotoxicity (Galloway et al., 2000). There are no specific data on DINP metabolites and their genotoxicity but the genotoxicity tests have been done in the presence of metabolic activation. There are several reports suggesting that H_2O_2 produced from overexpression of acyl CoA oxidase causes neoplastic conversion including growth in soft agar and formation of tumors in nude mice (Chu et al., 1995; Okamoto et al., 1997; Dadras et al., 1998). While this suggests that oxidative damage can lead to cell transformation in vitro, this has not been clearly demonstrated in vivo. It also of interest to note that chronic exposure to H_2O_2 causes gene amplification of catalase in a mammalian cell line (Hunt et al., 1998).

D. Conclusion

Collectively, the majority of data indicate that DINP is non-genotoxic (Doull et al., 1999), consistent with results obtained from analysis of other peroxisome proliferators (Doull et al., 1999; Ashby et al., 1994; Galloway et al., 2000; Lefevre et al., 1994; Gonzalez et al., 1998). DINP has been tested in bacterial mutation assays and mammalian gene mutation assays *in vitro*, with or without metabolic activation, and found to be non-mutagenic. DINP has also been evaluated in both *in vivo* and *in vitro* cytogenetic assays with results supporting the idea that DINP is not genotoxic. Lastly, *in vitro* analysis of unscheduled DNA synthesis in rat hepatocytes, which are known target cells of peroxisome proliferators, provided no evidence of mutagenicity caused by DINP.

Table VIII-1. Results of DINP Genotoxicity Assays

Assay	Result	Reference
Ames assay	–	BASF 1986, 1995; EG&G Mason Research, 1980; Exxon, 1996a; McKee et al., 2000; NTP, 1983; Zeiger et al., 1985
Mammalian DNA damage assay (rat hepatocytes) Unscheduled DNA synthesis	–	Litton, 1981
Mammalian mutation assay L5178Y mouse lymphoma cells	–	Barber et al., 2000; Hazelton, 1986
Mammalian chromosome damage assay		
In vitro rodent chromosomal aberrations	–	Exxon, 1996b; McKee et al., 2000
In vivo rodent micronucleus	–	McKee et al., 2000
In vivo cytogenetics	–	Microbiological Associates, 1981
Mammalian cell transformation	–	Barber et al., 2000

E. References

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IX. EVIDENCE ON DINP CARCINOGENICITY

A. Introduction

This section reviews the evidence on DINP carcinogenicity from chronic animal studies. No epidemiological studies of DINP carcinogenicity are available. Conclusions regarding the carcinogenicity of DINP in humans are therefore based on animal bioassays of DINP and structurally related compounds, and on investigations of the mechanisms of DINP carcinogenesis in animals. The mechanistic data are reviewed in Section X, and conclusions made regarding the human carcinogenic potential of DINP in Section XI.

Dietary carcinogenicity studies were conducted by Covance Laboratories for the Aristech Chemical Corporation (Moore, 1998a) in Fischer 344 rats and B6C3F1 mice (Moore, 1998b) of both sexes, and by Exxon Biochemical in Fischer 344 rats (Lington et al., 1997). Dosing was initiated in young adult animals. The design and results of these studies are described in detail below.

The section also compares DINP carcinogenicity findings to those for other phthalates. Of particular interest are the studies in male and female Sprague Dawley rats of the Monsanto material Saniticizer 900, a pure (99.9%) mixture of dinonylphthalates. These studies are therefore reviewed at length.

B. Studies of Technical Grade DINP in Rats

Covance Laboratories

Diets containing DINP at levels 0, 500, 1500, 6000, or 12000 ppm were fed to male and female Fischer 344 rats for at least 104 weeks. Additional animals added to the control and top two dose groups were sacrificed at 1, 2, 13, and 79 weeks. To study recovery, additional animals of both sexes received 12000 ppm for 72 weeks, and then basal diet for 28 weeks. Positive controls received for 13 weeks diets containing 1000 ppm Wy 14643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]-acetic acid). The liver, testes with epididymides, uterus, spleen and kidneys from animals scheduled for sacrifice after treatment for 78 or 104 weeks were examined microscopically. Microscopic examination of other identified remaining tissues was limited to control and high dose animals and all other animals with unscheduled deaths during the study.

Test animals were supplied by Charles River Laboratories, Raleigh, North Carolina. The test material, reported to be greater than 99% pure, was supplied by the test sponsor, Aristech Chemical. The study was conducted at the Covance Laboratories facility in Vienna, Virginia. Dosing was initiated in May, 1992.

The Consumer Product Safety Commission extracted individual animal data from the Covance report – time of death and the presence and type of liver, kidney and splenic tumor. The National Toxicology Program (NTP) kindly computed age-adjusted and non-age adjusted incidence values and tests for statistical significance for these data. Complete results of the NTP analysis are provided in Appendix B, section A.

Hepatocellular neoplasia was elevated for high dose animals of both sexes, with dose-related trend statistics highly significant (Table IX-1). Because these tumors were not observed to be lethal in this study, the table below provides statistics calculated using logistic regression. Results are similar to those obtained using the poly-3 test. For alternative calculations, see Appendix B Tables B5-B8. Hepatocellular carcinoma was first observed in high dose males at the interim sacrifice and in other dose groups at the terminal sacrifice.

Table IX-1. Incidence [a] of hepatocellular neoplasia in Fischer 344 rats chronically administered DINP in feed (Covance [Moore, 1998a])

	Concentration in feed (ppm)				
	0	**500**	**1500**	**6000**	**12000**
Males					
Carcinoma					
overall incidence	1/65	0/50	0/50	1/65	12/65
interim sacrifice	0/10	NA	NA	0/10	1/10
statistics	p<0.001	–	–	–	p<0.001
Carcinoma or adenoma					
overall incidence	5/65	3/50	2/50	7/65	18/65
interim sacrifice	1/10	NA	NA	0/10	1/10
statistics	p<0.001	–	–	–	p<0.001
Females					
Carcinoma					
overall incidence	1/65	0/49	0/50	1/65	5/65
interim sacrifice	0/10	NA	NA	0/10	0/10
statistics	p=<0.002	–	–	–	p=0.086
Carcinoma or adenoma					
overall incidence	1/65	1/49	0/50	2/65	8/65
interim sacrifice	0/10	NA	NA	0/10	1/10
statistics	p<0.001	–	–	–	p=0.018

[a] Where results are of borderline significance or greater, level of statistical significance computed by logistic regression is given. Significance value for trend is given in the column for the control group. Significance values for these findings calculated using different statistical tests are given in Appendix B, section A.

Study authors noted that histological and biochemical analyses indicated hepatocellular proliferation in animals sacrificed during the first week of the study and not at later observation time points. At later time points, the mean value for palmitoyl-CoA oxidase activity, an indicator of peroxisome proliferation, significantly increased in males and

females in the high dose group. In addition to neoplasia, liver histological findings noted as increased in high dose group animals in comparison with untreated controls included: diffuse hepatocellar enlargement (first detected during the second week), cytoplasmic eosinophilia (first detected at week 13), spongiosis hepatis and increased pigment in Kupffer cell canaliculi (first detected at week 79).

Renal tubular carcinomas were observed in four recovery group males and at the terminal sacrifice in two high dose males (Table IX-2). Other renal lesions observed histopathologically at the interim sacrifice and study termination included a dose-related increase in renal papilla in males, and a dose-related increase in the severity of tubule cell pigment in both sexes. Statistically significant increases in the mean of the relative as well as absolute kidney weight also were observed in both sexes at the interim sacrifice and study termination.

Table IX-2. Incidence of renal tubular carcinoma in male Fischer 344 rats chronically administered DINP in feed (Covance [Moore, 1998a])

	Concentration in feed (ppm)					
Incidence	**0**	**500**	**1500**	**6000**	**12000**	**12000 Recovery**
overall	0/65	0/55	0/55	0/65	2/65	4/50
at interim sacrifice	0/10	NA	NA	0/10	0/10	–
statistics	p=0.022[a]	–	–	–	p=0.219[a]	p=0.03[b]

[a] Level of statistical significance computed by poly-3 method, since it could not be done by logistic regression. The trend value, given in the column for the control group, does not include the recovery group. Significance values for these findings calculated using different statistical tests are given in Appendix B, section A.

[b] Level of statistical significance computed by Fisher Exact test. (Statistical tests were not run by NTP on recovery group.)

Treatment related increases in the incidence of mononuclear cell leukemia (MCL) were observed in both male and female rats, as shown in Table IX-3 below. Because MCL is relatively lethal (Ward et al., 1990), life table statistical tests are preferred over logistic regression, correspondingly p-values from life table analyses are reported below. MCL was first observed at study day 352 in the male 6000 ppm dose group. With the exception of the control male group, these relatively lethal neoplasia were first observed histologically in other dose groups from unscheduled necropsies. The statistical analyses comparing treated and control animals indicate the MCL to be treatment-related. However, this has been questioned because historically control values can differ substantially from study to study. This issue is discussed at greater length in section E below.

As is common for the Fischer 344 rat, benign testicular interstitial cell tumors were found in nearly all control and treated animals. For this reason, this study can be seen as an inadequate test for neoplasia of the testis. Because the testis is a target organ for phthalates, this is a significant study limitation.

Table IX-3. Incidence [a] of mononuclear cell leukemia in Fischer 344 rats chronically administered DINP in feed (Covance [Moore, 1998a])

Incidence	Concentration in feed (ppm)				
	0	500	1500	6000	12000
Males					
overall incidence	22/65	23/55	21/55	32/65	30/65
at interim sacrifice	1/10	NA	NA	0/10	0/10
statistics	p=0.002	–	–	p=0.027	p=0.022
Females					
overall incidence	17/65	16/49	9/50	30/65	29/65
at interim sacrifice	0/10	NA	NA	1/10	1/10
statistics	p<0.001	–	–	p=0.020	p=0.021

[a] Where results are of borderline significance or greater, level of statistical significance computed by life table analysis is given, since MCL is a relatively lethal disease. Significance value for trend is given in the column for the control group. Significance values for these findings calculated using different statistical tests are given in Appendix B, section A.

Lington et al., 1997

Groups of Fischer 344 rats of both sexes were fed diets containing 0, 300, 3000 or 6000 ppm DINP for up to 24 months. Interim sacrifices were scheduled at 6, 12, and 18 months. The study was terminated at 24 months. The animals were approximately 6 weeks old at study initiation. Microscopic examination was limited to all major organs from control and high dose animals plus gross lesions and kidney and liver tissue from mid- and low- dose animals. The supplier of test animals was Charles River Breeding Laboratory, Stoneridge, New York. The supplier of the test substance (CAS No. 68515-48-0), characterized as Jayflex DINP, was the Exxon Chemical Company, Baton Rouge. The test material was reported to be greater than 99% pure. Isononyl alcohol was noted as a known impurity of DINP, but concentrations in the test material were not given. The test was conducted at the Exxon Biochemical facility in East Millstone, New Jersey.

Unlike observed in the Covance study, liver carcinoma incidence was elevated in high dose males only, and the test for trend was significant (Table IX-4). Liver cancers were not significantly elevated in female animals. The combined incidence of neoplastic nodules and carcinoma was not elevated in either males or females. At the 18-month and terminal sacrifice, slight centrilobular to midzonal hepatocellular enlargement was observed and can be noted as dose-related.

Tubular cell kidney carcinomas were observed in one male in the lowest dose group and two males in the highest dose group (p=0.10, Fisher exact trend test). Transitional cell carcinomas of the kidney were observed in three mid-dose males. While these findings are of marginal statistical significance when compared with control animals, the occurrence of such tumors in Fischer 344 rats is uncommon (Boorman et al., 1990). In

high-dose males, a minimal increase in tubular cell pigment in the tubular epithelium was noted.

Table IX-4. Incidence [a] of hepatocellular lesions in Fischer 344 rats chronically administered DINP in feed (Lington et al., 1997)

Lesion	Concentration in feed (ppm)			
	0	300	3000	6000
Males				
Hepatocellular enlargement	1/81	1/80	1/80	9/80*
Carcinoma [b]	0/81	0/80	0/80	3/80**
Neoplastic nodules or carcinoma	3/81	1/80	1/80	4/80
Females				
Hepatocellular enlargement	1/81	1/81	0/80	11/80***
Carcinoma	1/81	0/81	0/80	1/80
Neoplastic nodules or carcinoma	1/81	2/81	0/80	2/80

[a] Fisher exact test for pairwise comparison between treated and control: *p=0.008; **p=0.12; ***p=0.002.
[b] p=0.0.15 for Fisher exact trend test.

Dose-related increases in the incidence of mononuclear cell leukemia were observed in both sexes (Table IX-5). The relevance of these findings in light of modulating incidence rates for MCL among control groups is discussed in detail in Section E.

As with the Covance study, interstitial cell tumors of the testis were observed in nearly all control and treated males. For this reason, this study can be seen as insensitive for determining the possible neoplastic impact of exposure to DINP on the testis.

Table IX-5. Incidence [a] of mononuclear cell leukemia in Fischer 344 rats chronically administered DINP in feed (Lington et al., 1997)

	Concentration in feed (ppm)			
	0	300	3000	6000
Males	33/81	28/80	48/80	51/80
	p=0.00003	–	p=0.011	p=0.0028
Females	22/81	20/81	30/80	43/80
	p=0.00001	–	p=0.11	p=0.0005

[a] Statistics for pairwise comparison of treated and control incidences by the Fisher exact test are given beneath incidence values for treated animals. Statistics for trend tests are given beneath control incidences. Cochran-Armitage and exact trend tests resulted, to one significant figure, in the same value, given in the table.

C. Studies of Technical Grade DINP in Mice

Covance Laboratories

The study protocol is similar to that described above for rats. Diets containing DINP at levels 0, 500, 1500, 4000, or 8000 ppm were fed to male and female B6C3F1 mice for at least 105 weeks. Additional animals added to all dose groups were sacrificed at 1, 2, 13, and 79 weeks. To study recovery, additional animals of both sexes received 12000 ppm for 78 weeks, and then basal diet for 26 weeks. Test animals were at least 6 weeks old at the initiation of dosing in September, 1993. Microscopic examination of tissues from nearly all animals scheduled for sacrifice after treatment occurred for liver, spleen, kidney, testis with epididymides (males), and uterus (females). Microscopic examination of other identified remaining tissues was limited to control and high dose animals and all other animals with unscheduled deaths during the study. Test animals were supplied by Charles River Laboratories, Portage, Michigan. The test material, reported to be greater than 99% pure, was supplied by the test sponsor, Aristech Chemical. The study was conducted at the Covance Laboratories facility in Vienna, Virginia.

As in the case of the Covance study in rats, the Consumer Product Safety Commission extracted individual animal data from the bioassay report and the NTP computed age-adjusted and non-adjusted incidence values and tests for statistical significance for these data. Complete results of the NTP analysis are provided in Appendix B, section B.

A dose-related increase in malignant and benign and malignant liver tumors occurred (Table IX-6). The table below provides statistics calculated using logistic regression. Results are similar to those obtained using the life table and poly-3 tests (See Appendix B, section B). In males and females, hepatocellular carcinoma is significantly increased above control levels in the top two dose groups. The combined incidences of carcinoma and adenoma are increased as well – in females at all but the lowest dose, and in males unequivocally in the top two dose groups. Dose related increases in metastases to the lung were observed in males; metastases to the lung were observed in six treated females, without a strong dose-related trend. Hepatocellular carcinoma was first observed in high dose males at study day 366, and in high dose females at study day 537. The latest observations of this tumor type were for control animals – at day 656 for males and the terminal sacrifice for females. Most animals found to have liver tumors were observed with liver tumor at scheduled sacrifice.

Significant increases in absolute and relative liver weight means occurred in the top two dose groups of both sexes at the interim sacrifice, and the same was found for all but the lowest dose group at the terminal sacrifice. Non-neoplastic histopathological findings for the liver that appeared dose-related included diffuse hepatocellular enlargement, increased cytoplasmic eosinophilia, pigment, and focal necrosis. As a further indication of liver toxicity, the authors note increases in serum alanine aminotransferase and aspartate aminotransferase.

Table IX-6. Incidence [a] of hepatocellular neoplasia in B6C3F1 mice chronically administered DINP in feed (Covance [Moore, 1998b])

Incidence	Concentration in feed (ppm)				
	0	**500**	**1500**	**4000**	**8000**
Males					
Carcinoma					
overall	10/70	8/67	10/66	17/65	20/70
interim sacrifice	0/15	0/14	1/13	2/14	3/15
statistics	p<0.001	–	–	p=0.057	p=0.019
Carcinoma or adenoma					
overall	16/70	13/67	18/66	28/65	31/70
interim sacrifice	1/15	1/14	4/13	3/14	4/15
statistics	p<0.001	–	–	p=0.007	p=0.002
Females					
Carcinoma					
overall	1/70	2/68	5/68	7/67	19/70
interim sacrifice	0/15	1/15	0/14	0/14	2/15
statistics	p<0.001	–	p=0.109	p=0.025	p<0.001
Carcinoma or adenoma					
overall	3/70	5/68	10/68	11/67	33/70
interim sacrifice	0/15	1/15	1/14	0/14	3/15
statistics	p<0.001	–	p=0.041	p=0.012	p<0.001

[a] Where results are of borderline significance or greater, level of statistical significance computed by logistic regression is given. Significance value for trend is given in the column for the control group. Significance values for these findings calculated using different statistical tests are given in Appendix B, section B.

Study authors report that serum and urine chemistry at weeks 26, 56, 78 and 104 indicate the kidney to be a target of DINP toxicity. The authors conclude that there may have been a treatment-related alteration in the concentrating ability of the renal tubule epithelium, possibly due to chronic progressive nephropathy. Dose related decreases in absolute and relative kidney weights were also reported for males. No tumors originating from the kidney were reported.

At the terminal sacrifice, decreased testicular weight was reported for all but the lowest dose group, but no histological correlates were noted. Two high dose females were observed with pancreatic islet carcinoma; none of the control animals was observed with these tumors.

D. Carcinogenicity Findings for Related Materials

This section briefly reviews the results of carcinogenesis studies on phthalates related to DINP. Included in this discussion is a relatively lengthy review of the bioassay on the

dinonylphthalate mixture Saniticizer 900. It is notable that a significant fraction of animals in dinonylphthalate bioassays were found with spongiosis hepatis, a lesion characteristic for DINP not routinely seen with other phthalates

Biodynamics (1986) study of pure (99.9%) dinonylphthalate

Groups of 70 male and female Sprague-Dawley CD rats were fed ad libitum doses containing dinonylphthalate for up to 2 years at nominal concentrations of 0, 500, 5000, or 10,000 ppm. At the end of the first year 10 animals of each sex in each treatment group were killed. The remaining surviving animals were killed at 2 years. Histological evaluations of tissues from 44 selected tissues (all major organs included) were conducted for all animals in control and high dose groups, and of liver tissue in all animals in the study.

Test animals were supplied by Charles River Breeding Laboratories, Portage, Michigan. The test material (CAS No. 71549-78-5), supplied by Monsanto, was reported to be 99.9% pure, with trade name Saniticizer 900. The study, sponsored by Monsanto, was conducted at the Biodynamics facility in East Millstone, New Jersey. Dosing was started in October 1981.

Mean intake levels calculated by the laboratory were 27, 271, and 553 mg/kg-day for males and 33, 331, and 672 mg/kg-day for females in low, medium, and high dose groups. Mortality was not reduced by treatment.

Liver lesions observed in the study included dose related increases in spongiosis hepatis in both sexes and minimal to slight focal necrosis in treated males (Table IX-7). With respect to liver neoplasia, a significantly increased incidence of hepatocellular carcinoma was observed in high and mid-dose females, and elevations above control levels of these tumors in high- and mid-dose males.

Historical control statistics for 13 chronic studies conducted by Biodynamics on Sprague Dawley rats supplied by Charles River are given in Table IX-8. The chronic studies were conducted between December 1979 and April 1981. Because the incidence of hepatocellular carcinoma in mid- and high-dose females fell outside the historical control range, the study authors concluded the findings in the female rat were dose-related. The incidence of carcinoma in mid- and high- dose males also falls outside the historical control range.

Both testicular cell hyperplasia and tumors were elevated above concurrent controls in high dose treated rats, as shown in Table IX-9. Testis from low- and mid- dose animals were not histologically evaluated for this tumor type. Table IX-9 also provides historical control data for testicular interstitial cell hyperplasia and tumors. The data were compiled from 14 chronic carcinogenicity studies conducted between December 1979 and April 1981 by Biodynamics on Sprague Dawley rats supplied by Charles River. There is a clear increase in testicular hyperplasia associated with dinonylphthalate treatment. Testicular tumor incidences in high dose animals are significantly above those

Table IX-7. Liver lesions in Sprague Dawley rats treated with dinonyl phthalate in feed (Bio/dynamics, 1986)

	Males ppm in feed				Females ppm in feed			
	0	**500**	**5000**	**10000**	**0**	**500**	**5000**	**10000**
spongiosis hepatis	16/70 (23%) p<0.001	11/69 (16%) –	30/69 (43%) p=0.008	32/70 (46%) p=0.004	4/70 (5.7%) p<0.01	3/70 (4.3%) –	6/70 (8.6%) –	11/70 (15.7%) p=0.049
necrosis	5/70 (7.1%)	17/69 (25%)	11/69 (16%)	23/70 (33%)	10/70 (14.3%)	15/70 (21.4%)	7/70 (10%)	10/70 (14.3%)
neoplastic nodules	2/70 (2.9%)	5/69 (7.2%)	6/69 (8.7%)	5/70 (7.1%)	1/70 (1.4%)	1/70 (1.4%)	5/70 (7.1%)	2/70 (2.9%)
hepatocellular carcinoma	2/70 (2.9%) p=0.15	2/69 (3.3%) –	6/69 (8.7%) p=0.13	4/70 (5.7%) –	0/70 (0) p=0.0004	0/70 (0) –	5/70 (8.3%) p=0.029	7/70 (10%) p=0.007

[a] Statistics for pairwise comparison of treated and control incidences by the Fisher exact test are given beneath incidence values for treated animals. Statistics for exact trend tests are given beneath control incidences.

Table IX-8. Historical control data for Biodynamics studies in Sprague Dawley rats supplied by Charles River

Historical Control Incidence		Neoplastic Nodules		Hepatocellular Carcinoma	
		Males	Females	Males	Females
Combined from untreated controls in 13 studies		96/1144 (8.4%)	103/1143 (9%)	36/1144 (3.1%)	18/1143 (1.6%)
Range:	low	0/117 (0%)	0/112 (0%)	0/56 (0%)	0/117 (0%)
	high	37/118 (31.3%)	40/119 (34%)	6/117 (5.1%)	6/119 (5%)

in the controls, but fall within the range of historical control values. Still the clear finding of interstitial cell hyperplasia suggests that these tumors are treatment-related.

Lesions that were elevated in uterine tissue are noted in Table IX-10. Tissue from mid- and low- dose groups were not examined. The incidence of endometrial hyperplasia was several fold greater in treated than control animals (p=0.002, Fisher exact test), and two adenocarcinomas were observed in treated animals. Historical control values were not found in the bioassay report, although it has been noted that spontaneous uterine neoplasms are rare in Sprague Dawley rats (Anisimov and Nikonov, 1990).

Urinary tract tissues from mid- and low-dose animals were not examined. Hyalin droplets in the convoluted tubules were observed in two high dose males and three control females. Medulla mineral deposits were observed elevated in treated males (25/70 versus 3/70 in controls; p=0.000002). Histopathological findings for the urinary bladder included an increase in urothelial hyperplasia in high dose males (6/68 versus 2/70 in controls, p=0.13). One bladder urothelial carcinoma was observed in the study in a high dose male and renal cortical neoplasia were observed in three control and one treated male, and one control female. These were the only tumors of the urinary tract observed.

Two other lesions in this study are worthy of note. In high dose males, pancreatic islet cell carcinoma was elevated four-fold (4/70 versus 1/70 in controls; p=0.2, Fisher exact). Historical values for this tumor were not reported. In one study of spontaneous incidence of tumors in Sprague Dawley rats these were rare (1/1340 males; Chandra et al., 1992). Also elevated in this dose group was the incidence of hyperplasia of the parathyroid (29/62 versus 19/56 in controls; p=0.11, Fisher exact), along with a slight elevation in adenoma (5/68 versus 2/66, p=0.23). As noted by study authors, the significance of these findings is uncertain.

To summarize, dinonylphthalate is hepatocarcinogenic to female Sprague Dawley rats, findings were equivocal in the male. It is of interest that effects were not observed at hematopoietic sites. Further study may reveal activity at extrahepatic sites. The study

suggests potential activity in the testis, endometrial tissue, and perhaps pancreas and parathyroid.

Table IX-9. Testicular hyperplasia and tumors in Sprague Dawley Rats treated with dinonyl phthalate in feed (Bio/dynamics, 1986)

Testicular lesion:	Historical controls	Concurrent controls	High dose group
Interstitial cell hyperplasia	17/1185 (0/118 – 3/42) (1.4% [0 – 7.1%])	4/59 (6.7%)	22/60[a] (36.6%)
Interstitial cell tumor	116/1185 (4/116 – 27/115) (9.8% [3.4 – 23.4%])	2/59 (3.4%)	7/60 (14%)

[a] Fisher exact values for pair-wise comparison of treated and control animals:
Interstitial cell hyperplasia – treated vs. concurrent control, p=0.0005; overall historical control, $p<10^{-20}$; highest finding in single historical control group, p=0.0004
Interstitial cell tumor – treated vs. concurrent control, p=0.09; overall historical control, p=0.38.

Table IX-10. Uterine lesions in Sprague Dawley Rats treated chronically with dinonyl phthalate in feed (Bio/dynamics, 1986)

Lesion	Concurrent controls	High dose group[a]
Endometrial colagenization/ fibrous thickening	5/70	13/69 p=0.035
Endometrial gland hyperplasia	2/70	13/69 p=0.002
Endometrial adenocarcinoma	0/70	2/69 p=0.25

[a]Fisher exact p-values of statistical significance of pair-wise comparisons between control and treated animals are also given.

Other Phthalates

Dietary exposure to diethylhexylphthalate (DEHP) was hepatocarcinogenic in F344 rats (NTP, 1982a; Rao et al., 1990; David et al., 1999) and B6C3F1 mice (NTP, 1982a; David et al., 1999) of both sexes. In mice and rats of the same strain, increased liver cancer was not seen for diallylphthalate (NTP, 1983, 1985), although for butyl benzyl phthalate in rats of both sexes (NTP, 1982b) there is a marginally significant dose response trend ($0.05 > p > 0.1$). Liver tumors were not observed in male Sprague Dawley rats fed 2% DEHP in diet for 102 weeks (Ganning et al., 1991). Interestingly, as noted by CPSC (1998) the potency of DINP and DEHP are similar, suggesting a key feature may be the presence of a long branched chain rather than specific isomer.

As noted above, pancreatic islet cell carcinomas were observed in male Sprague Dawley rats treated with dinonylphthalate, and in two high dose female mice treated with technical grade DINP. Four of fourteen Fischer 344 rats fed diets containing 2% DEHP for two years were found with pancreatic islet-cell adenomas (Rao et al., 1990). In a more recent study, Fischer 344 rats treated with DEHP were not observed with pancreatic islet cell tumors, but pancreatic acinar cell adenoma incidence was increased (5/59 at 12500 ppm, compared to 0/60 in control animals [p=0.03]; David et al., 2000). Acinar cell tumors were also increased with butyl benzyl phthalate treatment in male Fischer 344 rats (NTP, 1997).

Significant increases in mononuclear cell leukemia were observed in both male and female Fischer 344 rats in both DINP studies. Increases in these tumors have also been seen in Fischer 344 rats treated with other phthalates. In the recent study with DEHP, increases were seen in males (David et al., 2000). NTP concluded butyl benzyl phthalate was "probably carcinogenic for female F344/N rats" based on increases in these tumors (NTP, 1982b). This finding was not confirmed in a later study (NTP, 1997). The incidence of these tumors was also observed to be increased in female animals treated with diallyl phthalate, but the finding was seen as equivocal (NTP, 1985).

In adult male Sprague Dawley rats exposed *in utero*, Leydig cell hyperplasia and adenoma were observed with dibutyl phthalate treatment (Mylchreest et al., 1999, 2000) and hyperplasia with DEHP treatment (Parks et al., 2000).

E. Discussion

In the Covance studies, DINP was observed to be hepatocarcinogenic to males and females in high dose groups of both rodent species. Liver tumors were not observed to be dose related in the Exxon studies, but the doses used were lower than used in the Covance studies. In both studies, treated male rats developed renal tubule carcinomas, which were clearly dose-related in one study (Covance). Dose-related increases in mononuclear cell leukemia were also observed in male and female rats in both laboratories. In the Bio/dynamics studies using a pure mixture of dinonylphthalate isomers and a different rat strain, hepatocellular carcinoma was induced in females, and may have been in males. DINP has not been tested for carcinogenicity in young rodents, an important limitation given that infants and toddlers are the ones most exposed to DINP.

The relevance to humans of the findings of liver and kidney cancers will be discussed in the context of the mechanisms of DINP carcinogenesis at these sites (See Sections X and XI). The findings of mononuclear cell leukemia have been questioned because of the variability in incidence in control Fischer rat groups, the high degree of occurrence in the Fischer rat, and concerns regarding the biological relevance to human cancer (Caldwell, 1999b). Due to the modulation of incidence by diet and other factors and changes in the control incidence with time, it is important in historical analyses to compare findings with controls from the same laboratory over the same period. Such historical data were not

available to the Panel. Still, mononuclear cell leukemia was found in both sexes in both studies of technical grade DINP, in each of the four cases with a very high degree of statistical significance. Also, while the lesion rarely occurs in untreated rats less than 20 months of age (Stefanski et al., 1990), DINP treated animals were first observed with this tumor at considerably younger ages (see above). It is therefore highly unlikely that these findings were unrelated to treatment. Mononuclear cell leukemia, sometimes referred to as "Fischer rat leukemia" or large granular lymphocyte leukemia, is seen in other rat strains, sometimes with relatively high spontaneous incidence, and large granular lymphocyte proliferative diseases occur in humans (Ward et al., 1990).

To summarize, DINP is clearly carcinogenic to the rodent, inducing hepatocellular carcinoma in rats and mice of both sexes, renal tubular carcinoma in male rats, and mononuclear cell leukemia in male and female rats, and the dinonylphthalate studies suggest possible carcinogenicity in the testis, uterus, and pancreas. The chemical has not been tested for carcinogenicity in young rodents, an important limitation given that infants and toddlers are the ones most exposed to DINP. Chronic carcinogenicity studies have not been conducted in non-rodent species. Because of the lack of confidence in the relevance of the DINP rodent studies to humans (Sections X and XI), studies in species believed to produce results of greater relevance are clearly needed.

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X. CARCINOGENIC MECHANISMS

This section discusses the mechanisms by which DINP may induce the cancers seen in the rodent bioassays. These were cancers of the liver in mice and rats, renal tubular carcinoma in male rats, and mononuclear cell leukemia in male and female rats.

A. Liver Cancer

Any consideration of the mechanism(s) of the hepatocarcinogenic action of DINP must include the possibility that DINP might exert its carcinogenic effect by its ability to induce peroxisome proliferation in liver parenchymal cells, provided that it is unequivocally established that DINP is indeed classifiable as a peroxisome proliferator. The term "peroxisome proliferation" currently refers to the increase in the number and volume of cytoplasmic organelles called peroxisomes in liver parenchymal cells. Any agent that induces peroxisome proliferation and associated pleiotropic responses is designated as a "peroxisome proliferator".

Peroxisomes are single-membrane-bound cytoplasmic organelles, which are widely distributed in most animal and plant cells. Although they contain more than 60 proteins and participate in many metabolic functions including lipid metabolism, it is essential that these organelles possess at least one H_2O_2-generating flavin oxidase together with the H_2O_2-degrading peroxisomal marker enzyme catalase to be designated as peroxisomes. Peroxisome number and volume density remain fairly constant under various physiological and pathological conditions and in liver parenchymal cells peroxisomes normally occupy less than two percent of the cytoplasmic volume. Following exposure to peroxisome proliferators, the number and volume density of these organelles increase remarkably in rat and mouse liver to the extent that they may occupy as much as 25% of the hepatocyte cytoplasmic volume.

Peroxisome proliferators include a broad spectrum of synthetic and naturally occurring compounds, such as certain lipid and cholesterol lowering chemicals (e.g., clofibrate, nafenopin, ciprofibrate, fenofibrate, gemfibrozil, Wy-14,643), leukotriene antagonists, plasticizers (e.g., di-(2-ethylhexyl)phthalate (DEHP), di-(2-ethylhexyl)adipate), herbicides, solvents, and the naturally occurring steroid dehydroepiandrosterone, among others (Reddy and Lalwani, 1983; Kawashima, et al., 1983; Gonzalez et al., 1998). Despite their structural diversity, the synthetic peroxisome proliferators, as a group, induce qualitatively predictable immediate and delayed pleiotropic responses in rats and mice. The immediate responses consist of hepatomegaly, proliferation of peroxisomes in liver parenchymal cells, and the induction of several hepatic enzymes, particularly those responsible for lipid metabolism. Delayed responses include the development of hepatocellular carcinomas in rodents, by as yet undefined mechanisms (Chen et al., 1994; James and Roberts, 1994; Rao and Reddy, 1996; Rusyn et al., 2000). DINP is one of many peroxisome proliferators that cause hepatocarcinogenicity in rodents (see e.g., Rao and Reddy, 1996; NTP, 1982, Kluwe et al., 1982; Cattley et al., 1987; Woodward, 1988).

Peroxisome proliferators, irrespective of their structural diversity, have been consistently found to be nonmutagenic (nongenotoxic) in that they do not interact with or damage DNA either directly or after metabolic activation (Butterworth et al., 1984; Rao et al., 1994; Warren et al., 1982). This led to the proposal that the development of liver tumors is attributable to sustained induction of peroxisome proliferation and other related alterations (e.g., induction of microsomal CYP4A mediated fatty acid ω-oxidation and the mitochondrial fatty acid β-oxidation enzyme systems) associated with oxidative stress and indirect mutation (Kasai et al., 1989; Takagi et al., 1990; Reddy et al., 1980; Popp, 1992). This proposed basis for the development of liver tumors raises some intriguing questions about cell/tissue as well as species specificity. One such implication is that the carcinogenicity in rats and mice exposed to peroxisome proliferators, such as DINP, should be manifested only in organs or cell types which display the immediate pleiotropic responses. The second implication is that if immediate responses are minimal or do not occur in the liver parenchymal cells of a particular species, such species are less likely to develop liver tumors on chronic exposure. Extensive evidence supports the general assumption that peroxisome proliferator-induced pleiotropic responses are maximal in the liver of both rats and mice. The development of liver tumors is consistent with the tissue-specific nature of the peroxisome proliferator effects in these species. Accordingly, DINP hepatocarcinogenicity in rats and mice can in some measure be mechanistically associated with its ability to induce peroxisome proliferation in the liver since like all known synthetic peroxisome proliferators, DINP is also nonmutagenic (nongenotoxic).

Peroxisome Proliferator-Activated Receptor-α

The mechanisms by which the structurally disparate peroxisome proliferators induce similar predictable pleiotropic responses in a tissue/cell specific manner, and the basis for their hepatocarcinogenicity has engendered considerable debate (Rusyn et al., 2000). Significant progress has been made during the past decade in understanding the mechanisms responsible for the induction of several genes associated with the immediate pleiotropic responses, including those responsible for the peroxisomal, microsomal, and mitochondrial fatty acid oxidation systems in liver. The existence of a specific receptor(s) responsible for the action of peroxisome proliferators was first proposed in 1983 based on the cell/tissue specificity of pleiotropic responses, rapid transcriptional activation of fatty acid oxidation system genes, and response of extrahepatic hepatocytes to the inductive effects of peroxisome proliferators (Reddy and Lalwani, 1983). This formed the impetus for the identification and cloning of a receptor, now known as peroxisome proliferator-activated receptor-α (PPARα) from mouse liver (Issemann and Green, 1990), and the demonstration of its activation by structurally diverse peroxisome proliferators. The induction of some of the critical enzymes of the peroxisomal, microsomal, and mitochondrial fatty acid oxidation systems by peroxisome proliferators is transcriptionally controlled by PPARα: These pleiotropic effects, including the development of liver tumors, are abrogated in PPARα null mice (Lee et al., 1995; Peters et al., 1997a; Ward et al., 1998). Thus, the immediate pleiotropic responses, as well as the delayed hepatocarcinogenic effects, are directly attributable to PPARα activation by peroxisome proliferators. There is considerable evidence to support the hypothesis that sustained activation of PPARα and the resulting downstream metabolic perturbations

leading to intrahepatic oxidative stress, along with other effects of PPARα-activation (Roberts et al., 2000; Rusyn et al., 2000; Gonzalez et al., 1998), are responsible for the hepatocarcinogenicity of peroxisome proliferators (Roberts, 1996; Rao and Reddy, 1996; Gonzalez, 1998; Corton et al., 2000; Holden and Tugwood, 1999; Roberts et al., 2000). Since DINP induces peroxisome proliferation (Ashby et al., 1994; Barber et al., 1987; Lin, 1987; Moore, 1998a,b), is non-genotoxic (see Section VIII), and induces liver tumors, it is reasonable to classify this as a peroxisome proliferator and attribute its hepatocarcinogenity to PPARα-activation.

As indicated above, the term "peroxisome proliferator" was introduced to designate structurally diverse compounds that induce the classical phenomenon of peroxisome proliferation in the livers of responsive species. Accordingly, the receptor that is activated by peroxisome proliferators to mediate the typical pleiotropic responses has been appropriately called peroxisome proliferator activated receptor (PPAR). Although this PPAR subfamily of nuclear receptors has three isotypes, namely PPARα, PPARγ, and PPARβ/δ, it is now firmly established that peroxisome proliferation and liver tumors are induced by PPARα, and not the other two isotypes. The induction of peroxisome proliferation is associated with transcriptional activation of genes encoding for the peroxisomal β-oxidation system, and cytochrome P450 CYP4A isoforms CYP4A1, and CYP4A3 among others. For this to occur, PPAR heterodimerizes with retinoid X receptor (RXR), and this PPAR-RXR complex binds to PPAR-response element (PPRE), a region consisting of a degenerate direct repeat of the canonical AAGGTCA sequence separated by one base pair (DR1), present in the 5'-flanking region of target genes. The molecular mechanisms by which nuclear receptors, including PPARs, achieve transcriptional activation in a gene-, tissue-, and species-specific fashion are not fully understood. The current paradigm calls for the participation of additional factors for mediating the interaction between nuclear receptors and the basal transcription machinery in a ligand-dependent fashion. During the past six years, several nuclear receptor-interacting proteins, termed coactivators or corepressors, have been identified. Some of the coactivators possess intrinsic histone acetyltransferase activity, indicating a role in chromatin remodeling. SRC-1, PBP, CBP/p300, PGC-1, and PRIP, have been identified as PPAR coactivators, but further work is necessary to delineate functional implications of these molecules in determining the tissue and species responses to peroxisome proliferators.

Oxidative Stress

At least two sources of reactive oxygen radical production contributing to oxidative stress from chronic treatment with peroxisome proliferators have been hypothesized. First, the peroxisomal fatty acyl-CoA oxidase, the rate limiting enzyme of the classical inducible β-oxidation system, increases 20- to 40-fold, accompanied by only minimal increases in the activity of peroxisomal catalase and decreased activity of glutathione peroxidase (Nemali et al., 1989; Badr, 1992; Thottassery et al., 1992; Lazarow, 1977: Furukawa et al., 1983) . This has been considered the major source of H_2O_2 in exposed rodent liver. Second, peroxisomal oxidases such as the peroxisomal urate oxidase can show a modest 2-3-fold increase in the activity.

There is only about a two-fold increase in catalase activity and the disproportionate increases in the levels of H_2O_2-producing and H_2O_2-degrading enzymes have been shown to be due to differential regulation of genes encoding them. It is also worth noting that long chain dicarboxylic acids formed from CYP4A mediated fatty acid ω-oxidation serve as substrates for peroxisomal fatty acyl-CoA oxidase and their metabolism further adds to intracellular H_2O_2 levels (Reddy and Hashimoto, 2001). Consequently, it was hypothesized that an imbalance between H_2O_2 production and its degradation could lead to an increase in H_2O_2-mediated oxidative damage that eventually causes carcinogenesis in the livers of treated animals (Reddy and Lalwani, 1983; Reddy, 1990; Reddy, et al., 1980). The undegraded H_2O_2 reacts with transition metals leading to the generation of the highly reactive hydroxyl radical. Excessive $OH^{\cdot}$ radical production and oxidatively damaged DNA in the form of 8-hydroxydeoxyguanosine (8-OHdG) have been detected in livers of rats exposed to different peroxisome proliferators. Both short-term and long-term treatment with various peroxisome proliferators such as perfluorinated compounds, phthalate ester plasticizers, clofibrate, simifibrate, and ciprofibrate resulted in increased levels of 8-OHdG (Kasai et al., 1989; Takagi et al*.,* 1990; Cattley and Glover, 1993). Furthermore, ^{32}P-postlabeling studies have also shown unidentified DNA adducts in the liver of rats treated with ciprofibrate suggesting oxidative injury (Randerath et al., 1991).

Support for a mechanistic relationship between peroxisome proliferation and hepatocarcinogenicity is provided, in part, by a concordance with the magnitude of hepatic peroxisome proliferation and liver tumor development (Lake et al., 1987; Ashby et al., 1994). In addition, when H_2O_2-generating peroxisomal fatty acyl-CoA oxidase is overexpressed in stably transfected cell lines, such cell lines underwent neoplastic transformation when treated with fatty acyl-CoA oxidase substrates and produced tumors when xenografted in nude mice (Chu et al., 1995; Okamoto et al., 1997; Dadras et al., 1998).

There is also evidence that raises questions regarding the extent that oxidative stress plays the sole role in the hepatocarcinogenesis of peroxisome proliferators, leading several investigators to conclude that other mechanisms are potentially as or more important (see e.g., Rusyn et al., 2000; Roberts, 1996; Marsman et al., 1988; Gonzalez et al., 1998). They note the lack of in vivo measurements of reactive oxygen species and inconsistent results of cancer studies in animals with altered oxidant status. No direct relationship was observed between acyl-CoA oxidase induction for Wy-14,643 and the considerably less potent DEHP (Marsman et al., 1988). Undetected (Soliman et al., 1997) or only modest increases (Conway and Popp, 1995) in indicators of oxidative injury are seen in response to peroxisome proliferators. Fairly poor correlation between the multiplicity of tumors and the formation of 8-hydroxy-2'-deoxyguanosine (Cattley and Glover, 1993) or no sign of the induction of this indicator of oxidative injury was seen after treatment with peroxisome proliferators (Hegi et al., 1990; Hayashi et al., 1994). Non-detectable (Handler et al., 1992) or only very small increases (Tamura et al., 1990) in hydrogen peroxide have been detected during several fold increases in CoA activity after exposure to peroxisome proliferators. Peroxisome proliferators have not been demonstrated to be initiators in two stage models of carcinogenesis (Popp and Cattley,

1993; Williams et al., 1987; Glauert and Clark, 1989). In young and old rats exposed to the Wy-14,643 and observed for the same period, peroxisome proliferation was roughly the same, while old rats had a several fold higher yield of grossly visible hepatic tumors (Cattley et al., 1991). In addition, there is an increasing body of evidence indicating that peroxisome proliferator modulation of cell regulatory processes plays a significant role in carcinogenesis, and that Kupffer cells are involved, as discussed below.

Peroxisome Proliferator Modulation of the Cell Cycle

Peroxisome proliferators are mitogenic to the liver, inducing hepatomegaly and hyperplasia. They also exhibit promoting activity in initiation-promotion assays (e.g., Ward et al., 1984), but in a fashion dissimilar to that of phenobarbital (Ward et al., 1983). The mechanism(s) by which peroxisome proliferators cause cell proliferation is the subject of ongoing research. The proliferation of liver cells in rodents is markedly increased in the first few weeks following the initiation of treatment, and there is good correlation between sustained increases in replicative DNA synthesis associated with hyperplasia and carcinogenicity of peroxisome proliferators (Rusyn et al., 2000; Marsman et al., 1988). Treatment with Wy-14,643 results in expression of genes associated with cell proliferation (as well as other genes that inhibit growth) (Ma et al., 1997). In PPARα-null mice, a mitogenic response is not induced with peroxisome proliferator treatment (Peters et al., 1997a), nor is there elevation of cell cycle proteins (Peters et al., 1998). Thus, any contribution of mitogenesis to the process of carcinogensis is mediated by PPARα.

Peroxisome proliferators also inhibit apoptosis, or programmed cell death (Roberts, 1996, 2000; James et al., 1998). This is also mediated by PPARα (Roberts et al., 1998; Christensen et al., 1998). The peroxisome proliferator nafenopin reduced the level of induction of apoptosis by transforming growth factor β and bleomycin (Christensen et al., 1998; Bayly et al., 1994), but was unable to prevent apoptosis induced by DNA synthesis inhibiting hydroxyurea and etoposide, indicating its influence on apoptosis may depend on the nature of the apoptotic stimulus (Bayly et al., 1994). Peroxisome proliferator induced apoptosis also explains findings of experiments comparing tumor yields between young and old rats. In the experiment noted earlier (Cattley et al., 1991), the strikingly greater numbers of liver tumors in old compared to young rats treated with Wy-14,643 could not be explained by either hepatocellular proliferation or peroxisome proliferation, since neither was exaggerated in older animals. In a similar experiment by a different laboratory, numerous tumors were observed in old (initially 57 weeks) fed nafenopin for 13 months compared to a few tumors in young, similarly exposed rats (initially 13 weeks) (Kraupp-Grasl et al., 1991).

The Role of Kupffer Cells

As reviewed by Rusyn et al. (2000, 2001) and Gonzalez et al. (1998), recently the possibility that Kupffer cells are involved in the hepatocarcinogenesis of peroxisome proliferators has been explored in a series of experiments. Peroxisome proliferators increase hepatocyte proliferation to a far greater extent *in vivo* than *in vitro*. It has been

proposed that mitogenic cytokines from nonparenchymal cells are involved in hepatocyte proliferation. Kupffer cells, the resident hepatic macrophages, are the predominant source in the liver of mitogens, including TNF-α. It has been established that Kupffer cells and TNF-α play a role in Wy-14,643 mitogenesis (Bojes and Thurman, 1996; Rose et al., 1997) and, in general, Kupffer cells appear to be responsible for hepatocyte proliferation by mechanisms involving TNF-α. However, TNF-α null mice are not refractory to peroxisome proliferator induced cell proliferation (Lawrence et al., 2001). This suggests that the role of Kupffer cells in the mechanisms underlying peroxisome proliferator induced hyperplasia involves cytokines/chemical mediators other than TNF-α. Peroxisome proliferators activate the transcription factor NF-κB (nuclear factor-κB), one of the main regulators of TNF-α, in an oxidant dependent fashion. When Kupffer cells were treated *in vitro* with Wy-14,643, superoxide production was induced in liver cells from wild type, but not NADPH oxidase-null, mice. Also, in studies with Wy-14,643, liver weight and cell proliferation were increased in wild type, but not NADPH oxidase-null, mice. Further, the activation of NF-κB and cell proliferation was inhibited in *in vivo* experiments in Sprague Dawley rats treated with an inhibitor of NADPH oxidase (diphenyleneiodonium) (Rusyn et al., 2001). Thus, NADPH in Kupffer cells appears to be a source of free radicals from treatment with peroxisome proliferators. PPARα does not appear to be required for the generation of hydroxyl radicals**,** since they were induced in PPARα knockout mice.

It is unclear how Kupffer cells, which do not contain PPARα (Peters et al., 2000), and parenchymal cells, which do, interact. Most recently Parzefall et al. (2001) separated parenchymal from non-parenchymal cells and treated them with Wy-14,643 and nafenopin. Acyl-CoA activity was increased in the purified parenchymal cells, but DNA synthesis was not. Further, parenchymal cells cultured in a medium conditioned from isolated Kupffer cells and treated with Wy-14,643 exhibited increased DNA synthesis. This supports the notion that cytokines released from Kupffer cells and PPARα are both required for the proliferative response and cancer. As noted by Cattley et al. (1998), it is likely that peroxisome proliferators cause liver cancer by the regulation of as yet undefined gene networks, with the PPARα/RXR complex a key component of the mechanism. The work on the role of Kupffer cells appears to be uncovering some other of the important elements.

Mechanism of Rodent Liver Cancer

There is overwhelming evidence that events downstream of PPARα activation lead to liver cancer in rodents. However, the relative roles of the possible, nonexclusive, downstream mechanisms – oxidative stress, apoptosis, cell proliferation, with or without Kupffer cell involvement – are unclear (see e.g., Rusyn et al. 2000, 2001; Yeldandi et al., 2000; Roberts et al., 2000; Corton et al., 2000).

PPAR activation in humans versus rodents

Species differences in hepatic response to peroxisome proliferators have been widely discussed (e.g., IARC, 1995; Ashby et al., 1994; Bentley et al., 1993; Lock et al., 1989;

Lake, 1995a and b). While long-term treatment with peroxisome proliferators results in hepatocarcinogenesis in rats and mice (Ashby et al., 1994; Bentley et al., 1993; Lake, 1995a; Reddy and Lalwani, 1983), other species appear resistant. For example, the peroxisome proliferators nafenopin and Wy-14,643 induced liver tumors in rats but not Syrian hamsters after 60 weeks exposure, suggesting lower sensitivity in the hamster (Lake et al., 1993). The time course of tumor development of the two chemicals differed in the rat, and it is possible that with lifetime observation tumorigenicity may have been observed in the hamster. Non-human primates have been discussed as refractory to peroxisome proliferator-induced hepatocarcinogenesis (Cattley et al., 1998); however primate experiments have comprised less than half the lifetime of the animal (e.g., Tucker and Orton, 1993) and typically used few animals, particularly studies in Old World species. Also, as noted by IARC (1995), epidemiological studies of users of cholesterol-lowering drugs (e.g., Law et al., 1994; Huttunen et al., 1994) have insufficient power to evaluate the risk for hepatocellular cancer. Thus evidence on human susceptibility to liver cancer from peroxisome proliferators comes primarily from species comparisons of short term responses, such as proliferation of peroxisomes in liver parenchymal cells, hepatomegaly and the induction of various hepatic enzymes, and of PPARα expression.

Species differences in the response to peroxisome proliferators have been demonstrated in short-term *in vivo* studies. In the hamster study described above, peroxisome proliferation as measured by palmitoyl-CoA oxidation and liver weight was increased in both the rat and hamster, but indicators of cell replication were substantially increased only in the rat. Rodents typically respond to these chemicals by peroxisome proliferation, increased hepatic cell replication, and large increases in hepatic expression of lipid metabolizing enzymes. Rats and mice are highly responsive to peroxisome proliferators, Syrian hamsters exhibit an intermediate phenotype, where under similar conditions guinea pigs (Osumi and Hashimoto, 1978; Elcombe and Styles, 1989; Choudhury et al., 2000), marmosets (Holloway et al., 1982; Tucker and Orton, 1993), rhesus monkeys (Holloway et al., 1982)**,** and dogs are observed to be less responsive, or non-responsive. Although clearly less sensitive than rats and mice to these changes (IARC, 1995), non-human primates and other non-rodent species (i.e., cats, rhesus monkeys, cynomologus monkeys, pigeons**,** and chickens) exposed subchronically to a peroxisome proliferator were observed to have increased liver weight, peroxisome proliferation, and other related effects (Reddy et al., 1984). These findings are indicative of dose-dependent differences in species sensitivity rather than lack of responsiveness. Similarly, large differences in peroxisome proliferation have also been observed following nafenopin exposure in the rat, Syrian hamster, marmoset, and guinea pig - with each species responsive, but to a different degree (Lake et al., 1989). Increased liver weight accompanied by little or no evidence of peroxisome proliferation has been observed in females rhesus monkeys treated with clofibrate, male rhesus monkeys treated with clofenapate, and cynomologus monkeys but not marmosets treated with clobuzarit (Tucker and Orton, 1993).

Liver biopsies from humans treated with gemfibrozil or fenofibrate do not show evidence of peroxisome proliferation (De La Iglesia et al., 1982; Blumcke et al., 1983; Gariot et al., 1987). However, one study of clofibrate showed a significant 50% increase in

peroxisome number and a nonsignificant 23% increase in peroxisome density (Hanefield et al., 1983) and changes in peroxisome number and morphology have been reported in patients treated with fibrate drugs (PDR**,** 2000). Other studies suggest no increase or minor increases in peroxisome proliferation after exposure to DEHP or fibrates (for review see Huber et al., 1996). However, human studies to date are limited by health status of controls and treated subjects, numbers of subjects, and various other factors. Overall an unequivocal enhancing effect on peroxisome proliferation has not been observed, and none of the human liver biopsy studies suggested increases of a magnitude commonly observed in rodent studies. In two recent reviews of the medical significance of PPARαs, it was asserted that PPARα does not induce peroxisomes in humans and that therefore the term peroxisome proliferator per se in a medical context is a misnomer (Vamecq and Latruffe, 1999; Roberts, 1999).

In vitro analysis also supports many of the observations made *in vivo*. Peroxisome proliferators cause increased replicative DNA synthesis, suppression of apoptosis, increased expression of marker mRNAs and proteins including peroxisomal acyl CoA-oxidase (ACO), and peroxisome proliferation in cultured rodent hepatocytes (Elcombe, 1985; Duclos et al., 1997; Cornu-Chagnon et al., 1995; Perrone et al., 1998; Goll et al., 1999; Elcombe et al., 1996; Bichet et al., 1990; Hasmall et al., 1999 and 2000). It is important to point out that while peroxisome proliferation is reported to occur in cultured rodent hepatocytes exposed to these chemicals, a simultaneous comparison of this effect to that found *in vivo* has not been reported.

In contrast to results obtained from cultured rodent hepatocytes, peroxisome proliferators do not increase cell proliferation in cultured guinea pig hepatocytes (Styles et al., 1988). In general, increased replicative DNA synthesis, suppression of apoptosis, increased expression of marker mRNAs and proteins including peroxisomal ACO, or peroxisome proliferation have not been observed in human and non-human primate hepatocytes treated with these chemicals *in vitro* (Elcombe, 1985; Duclos et al., 1997; Cornu-Chagnon et al., 1995; Goll et al., 1999; Elcombe et al., 1996; Bichet et al., 1990; Hasmall et al., 1999 and 2000). Yet significant dose-dependent induction of acyl-CoA oxidase activity has been observed in human hepatocytes treated with clofibrate and ciprofibrate (Perrone et al., 1998; Scotto et al., 1995) and treatment with perfluorodecanoic acid resulted in significant induction of peroxisomal density and increased acyl-CoA oxidase activity in human cells derived from glioblastoma (Cimini et al., 2000). In one study (Perrone et al., 1998), the percent of apoptotic human hepatocytes was increased by both drugs, but DNA synthesis was observed to be inhibited (Perrone et al., 1998), leading the authors to conclude that human cells are refractory to peroxisome proliferator induced hepatocarcinogenesis. Collectively, *in vivo* and *in vitro* data strongly support the idea that humans are more resistant to many of the effects induced by peroxisome proliferators in rodents. Nevertheless, marked reductions in serum lipids occurs in both rodents and humans exposed to peroxisome proliferators. Since this effect is mediated by the PPARα in mice (Peters et al., 1997b), this suggests that humans have a functional PPARα.

PPARα is activated by fatty acids, eicoanoids, and peroxisome proliferators such as Wy-14643, nafenopin and clofibrate. Endogenous ligands include many fatty acids, and fatty

acids derivatives, arachidonic acid derived prostaglandins and eicosanoids (Kliewer et al., 1997; Forman et al., 1997; Krey et al., 1997). In humans and rodents, fibrate drugs used in the treatment of hyperlipidemia are thought to activate PPARα in the liver. Human PPARα activation results in increased apolipoprotein A-II and lipoprotein lipase transcription, reduced apolipoprotein C-III, which is key to lowering serum triglycerides (Vu-Dac et al., 1995; Auwerx et al., 1996; Staels et al., 1997), as well as induction of fatty acid transport protein and acyl-CoA synthetase (Martin et al., 1997). Apolipoprotein C-III is a major component of very low density lipoproteins and inhibits lipoprotein lipase and clearance of lipoproteins by the liver. Unlike rodents, PPARα activation in humans is not commonly observed to result in peroxisome proliferation, although the extent to which this has and can be studied *in vivo* is limited.

Transient transfections show that human PPARα can transactivate PPRE reporter constructs suggesting that the human isoform is functional (Sher et al., 1993). Thus it is not surprising that PPREs have been described in human genes that are transcriptionally regulated by the PPARα in the rodent genome including human apo C-III (Hertz et al., 1995), lipoprotein lipase (Schoonjans et al., 1996), apo A-I (Vu-Dac et al., 1994), apo A-II (Vu-Duc et al., 1995), carnitine palmitoyltransferase-I (Mascaro et al., 1998) and acyl CoA oxidase (Varanasi et al., 1996). Since the hypolipidemic effects of peroxisome proliferators are mediated by the PPARα in rodents and possibly humans, humans express an apparently functional PPARα, and the human genome contains genes with PPREs, it is of great interest to determine why human cells appear relatively insensitive to peroxisome proliferation and related effects. One possible mechanism to explain this disparity is that there are differences in the intracellular levels or function of the expressed receptor. PPARα mRNA levels in human liver are less than one-tenth the levels in mice (Palmer et al., 1998). Further, guinea pigs liver contains significantly less PPARα compared to mice (Bell et al., 1998). Thus it is possible that the level of PPARα in the human liver may not be sufficient to activate target genes that are regulated in response to peroxisome proliferators in rodent models, yet capable of modulating genes involved in lipid homeostasis. While lower levels of PPARα may in part contribute to the species differences, expression of truncated or mutant PPARα have also been described (Palmer et al., 1998; Tugwood et al., 1996; Gervois et al., 1999; Sapone et al., 2000; Vohl et al., 2000). This suggests that mutant PPARα variants may also contribute to the lower sensitivity of humans to peroxisome proliferators. However, since some humans are responsive to fibrate therapy, the hypothesis that altered PPARα protein accounts for the species difference is likely not true in all circumstances.

Another hypothesis to explain the species difference in sensitivity to peroxisome proliferators is that some human target genes may contain mutations or polymorphisms in the DNA responsive element. For example, it is reported that the PPRE for the human acyl CoA oxidase gene is inactive in transiently transfected cells (Woodyatt et al., 1999). However, another group previously reported that this PPRE is capable of being transactivated in reporter assays (Varanasi et al., 1996) after clarifying the original reported PPRE sequence (Varanasi et al., 1998). Subsequently, using site directed mutagenesis of the rat ACO PPRE, it was shown that the human PPRE sequence is not capable of being transactivated in reporter assays (Lambe et al., 1999). Due to this

controversy, further analysis of PPREs of other PPARα target genes in humans should be evaluated. Lastly, differences in the formation of PPARα-RXRα/ACO PPRE complexes in rat and human cell extracts has also been suggested as a related hypothesis to explain the apparent species differences and deserves further investigation (Rodriquez et al., 2000).

It is clear from several investigators that humans possess a functional PPARα, and the human receptor is activated by xenobiotic pharmaceuticals and industrial chemicals such as DINP and DEHP. It is also clear that some of the genes they regulate differ from those regulated by rodent PPARα. What is less clear is the relative potency of phthalates to activate the human receptor compared to therapeutic agents and endogenous activators, and whether such activation would have hepatocarcinogenic effects in humans (Cattley et al., 1998; Rusyn et al., 2000). In precautionary remarks, it has been noted that, due to species differences in tissue distribution of PPARs, site concordance for PPAR-mediated effects across species is not necessarily expected (Melnick, 2001). It has also been noted that the interindividual variability in PPAR sequences coupled with the inducibility of PPARα expression by peroxiosome proliferators, glucocorticoids and nutritional factors suggest that there may be certain individuals who are at increased cancer risk from chemicals that activate PPARα (Vanden Heuvel, 1999).

B. Kidney Tumors

The renal tubular carcinoma observed in male rats exposed to DINP has been attributed to cytotoxicity resulting from accumulation of α2μ-globulin in the kidney (Caldwell et al., 1999). In this subsection this mechanism is discussed, and the formal criteria for evaluating this potential mechanism of carcinogenicity are applied. Cytotoxicity can produce compensatory, regenerative cell proliferation. Administration of chemicals causing regenerative proliferation first results in histopathological lesions, necrosis, and the release of enzymes into the serum that are specific to the tissue that is damaged. Cell proliferation occurs to replace damaged cells and stops when an equal number of cells exist to the number before the toxic insult, leading to no increase in number or size of the newly replicated cells. Increases in tissue-to-body weight that may occur are usually due to fatty infiltration or other effects secondary to the toxicity. Compensatory cell replication due to cytotoxicity may provide a growth stimulus to spontaneously occurring preneoplastic cells, allowing them to overcome normal growth regulation. Increased cell division may also induce genetic damage by increasing the number of genetic errors due to rapid cell division and reduced time for accurate DNA repair. Toxic by-products of cell necrosis (for example, peroxidized lipids or damaged DNA bases) (El Ghissassi et al., 1995) may become associated with newly replicating cells, leading them to be transformed.

The relationship of cytotoxic cell proliferation and organ-specific carcinogenesis recently has become strikingly clear for chemicals that induce α2μ-globulin nephropathy. This syndrome is produced by a variety of chemicals and is manifested by accumulation of α2μ -globulin in the kidney resulting in compensatory cell proliferation and renal tubular

tumors. α2μ-globulin is a low molecular weight protein (18,700 d) synthesized in the livers of male, but not female rats. It is endocrine dependent**,** appearing during sexual maturity. Following secretion from the liver, α2μ-globulin is filtered in the glomerulous and slowly hydrolyzed in the proximal tubule. There is a strict requirement that a chemical or a metabolite physically bind to the α2μ-globulin to produce nephropathy, although covalent, irreversible binding is not required. The site of binding is likely in the liver or blood. When the chemical-α2μ-globulin complex is filtered by the kidney, it accumulates in phagolysosomes in the proximal tubule region and produces cytotoxicity, which results in subsequent regenerative hyperplasia (Lehman-McKeeman, 1997). Several nongenotoxic chemicals, including *d*-limonene, unleaded gasoline, jet fuels and 1,4-dichlorobenzene produce sustained nephrotoxicity which may be the causative factor in their induction of renal cancer (Borghoff et al., 1990; Borghoff and Lagarde, 1993; Swenberg et al., 1992). Female rats, mice, and NBR rats do not accumulate α2μ - globulin in their kidneys, do not produce nephrotoxicity and regenerative hyperplasia, and do not develop renal tumors following chemical exposure. It has been postulated that since humans appear to lack this specific protein in the kidney, they may, therefore, be refractory to renal carcinogenesis by this mechanism. However, extreme care should be given before dismissing potential human carcinogenicity of a chemical based on the absence of evidence. Other human proteins may bind these or other chemicals and produce toxicity and regenerative hyperplasia, even in the absence of α2μ-globulin. Alternatively, humans may metabolize this class of chemicals uniquely and produce toxicities not predicted from rat data. Although it is clear that data from studies in the male F344 rat associated with α2μ-globulin nephropathy may not be appropriate for assessment of human risk for renal carcinogenesis, other toxicities occurring in alternative sex-species may appropriately be evaluated that are not related to α2μ - globulin nephropathies.

DINP produces kidney tumors in male rats after dietary exposure at 1.2% in the diet, but not at 0.6% in the diet (Caldwell, et al., 1999). Because the tumors occurred in male rats and not female rats or mice, the male rat-specific α2μ-globulin mechanism of action was postulated. Immunohistochemical analysis demonstrated the accumulation of this protein in male rat kidneys. An increase in cell proliferation was also detected by measurement of proliferating cell nuclear antigen (PCNA). Increased cell proliferation was isolated to regions of α2μ-globin accumulation. Histopathological evaluation demonstrated renal tubular hypertrophy and regeneration, consistent with the hypothesis that α2μ-globulin accumulation produces chronic cytotoxicity and cell proliferation, resulting in neoplastic transformation. Male rat specificity in tumor response, lack of genotoxicity, histopathology findings of cytotoxicity and regeneration, α2μ-globulin accumulation**,** and demonstrated cell proliferation strongly support the criteria for demonstrating α2μ-globulin mechanism (IARC, 1998). Therefore, the renal tumors in male rats at the high dose of DINP are assumed to be rat specific and are not used to predict human cancer risk.

C. Mononuclear Cell Leukemia

Some toxicologic pathologists view mononuclear cell leukemia (MCL) in the Fischer rat as a unique type of cancer and not induced *de novo* by compound administration, and disregard it. MCL is one of the most common background tumor types in this strain, and has been referred to as Fischer rat leukemia, large granular leukemia, and Tγ lymphocyte leukemia (Ward et al., 1990; Boorman, et al., 1990). Other rat strains rarely develop MCL and mice do not develop MCL. MCL is a proliferative disease of large granular lymphocytes, a subpopulation of lymphocyte that mediates natural killer and antibody dependent cell mediated cytotoxic activity (Reynolds, 1985). The human correlate to rat MCL is chronic Tγ lymphoproliferative disease, which represents the abnormal expansion of large granular lymphocytes (Reynolds and Foon, 1984). Patients are predominantly older males, and exhibit with lymphocytosis of predominantly Tγ lymphocytes with lymphocyte infiltration of the bone marrow and often spleen. The leukemia in F344 rats has been noted as morphologically, functionally and clinically similar, and consequently an experimental model for exploring treatment (Reynolds and Foon, 1984). In the rat, chemically-related increases in MCL exhibit advanced severity grades for this lesion in treated rats compared to controls (Boorman, et al., 1990).

Mononuclear cell leukemia was increased in both sexes of Fischer 344 rats in DINP studies performed in two different laboratories, with a high degree of statistical significance in each of the four cases (See Section IX). There was not consensus on the CHAP regarding whether these findings should be considered compound related. It was argued that the incidence in the treatment groups should be compared with the historical controls. However, the historical control values specific to the test facilities and time periods of testing were not made available to the committee, precluding comparison with the appropriate historical control data. There is wide variability in the incidence of this tumor in the National Toxicology Program (10-72%). A guideline to consider to evaluate whether increased tumors are chemically related is to see if the incidence in treated animals falls outside the control range estimated to occur 95% of the time (Haseman et al., 1990). For NTP studies, the mean incidence for MCL is 32.9%; SD 14.6%. Thus, the upper bound for the control range for MCL is 62.1%. DINP was the only phthalate ester associated with MCL at this level (64% at 0.6% in the diet for two years). On the other hand, findings in the different treatment groups did not exhibit the random variability suggested by this calculation. MCL was found in both sexes in both studies of technical grade DINP, in each of the four cases with a very high degree of statistical significance. Findings were significant, typically in the top two dose groups, and dose related trends were highly significant. Also, while the lesion rarely occurs in untreated rats less than 20 months of age (Stefanski et al., 1990), DINP treated animals were first observed with this tumor at considerably younger ages.

Nonetheless, the majority of the CHAP viewed this tumor as being of questionable significance, due to its high and variable background and possible strain specificity, and did not use it for risk prediction.

D. Conclusions

Peroxisome proliferators are a structurally diverse group of non-mutagenic chemicals that induce predictable pleiotropic responses including the development of liver tumors in rats and mice. These nonmutagenic chemicals interact variably with peroxisome proliferator-activated receptors (PPARs), which are members of the nuclear receptor superfamily**.** Evidence derived from PPARα gene disruption indicates that of the three PPAR isotypes (α, β/δ and γ), the isoform PPARα is essential for the pleiotropic responses induced by peroxisome proliferators including the development of hepatocellular carcinomas. DINP is classifiable as a hepatic peroxisome proliferator and in that regard the liver tumors developing in rats and mice chronically exposed to DINP can be mechanistically related to PPARα activation. There are clearly species differences in response to peroxisome proliferators such as DINP suggesting that humans may be less responsive to these chemicals compared to rodents. Further research is necessary to conclusively identify mechanisms underlying these differences and their potential relevance to human risk assessment. While the evidence is overwhelming that events downstream of PPARα activation lead to liver cancer in rodents, the relative roles of the possible, nonexclusive, downstream mechanisms – oxidative stress, apoptosis, cell proliferation, with or without Kupffer cell involvement – are unclear.

Criteria for supporting an α2μ-globulin mechanism of action for renal tumors (IARC, 1998) were applied and found to be met. The renal tumors in male rats at the high dose of DINP were therefore treated as rat specific and were not used to predict human risk. The mononuclear cell leukemia (MCL) in Fischer 344 rats was viewed of questionable significance due to its high and variable background and possible strain specificity and also was not used in human risk prediction.

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XI. RISK CHARACTERIZATION

A. DINP Risk Assessment for Non-cancer Endpoints

For DINP, several issues complicate the risk assessment for non-carcinogenic or systemic toxic effects. These issues include: the selection of the appropriate NOAEL for spongiosis hepatis, the appropriate adjustment or uncertainty factors for systemic toxicity endpoints, and whether additional safety factors should be used for missing data.

Spongiosis hepatis

Chronic effects in the liver included spongiosis hepatis, a focal degeneration of the perisinusoidal cells of the liver, in two lifetime feeding studies of DINP in rats, one by Lington et al. (1997) under the sponsorship of the Exxon Company and the other by Moore (1998) and colleagues sponsored by Aristech Chemical Corporation. Table XI-1 compares the designs of these two studies.

Table XI-1. Experimental design of long term feeding studies of DINP in rats

Design Features	**Lington et al. 1997 (sponsored by Exxon)**	**Moore 1998 (sponsored by Aristech)**
Number of dose groups	3 plus control	5 plus positive (Wy-14,643) male and negative control
Reversibility	No	Yes
Age at start	Not reported	6 weeks
No. rats/sex/group at start	110	70-85
Interim sacrifices	6, 12 and 18 months	1, 2, 13 and 79 weeks
Level of DINP in diet	0.03, 0.3 and 0.6%	0.05, 0.15, 0.6 and 1.2%
Test article	Same as commercial product (DINP - 1)	Same as commercial product (DINP - 1)
Number of liver sections	4-5/rat	1-2/rat
No. at terminal sacrifice (planned)	80/sex/group	50-55/sex/group

Although the two studies were similar in design, they did not replicate exposure levels except for the 0.6% dietary level. The most marked differences were in the numbers of rats at the start of the experiment, the number and timing of the interim sacrifices, and the number of liver sections taken for histopathological examination. The number of liver slices examined is likely to have affected the numbers of rats detected with focal lesions.

Some results of the two studies to consider in the risk assessment are compared in Table XI-2. Although groups in both studies received diets containing 0.6% DINP, the estimated dosages in the Moore study were greater than those of Lington et al. by 17 and 18% for male and female rats, respectively. Other notable differences were in the control incidences of rats with mononuclear cell leukemia and spongiosis hepatis.

Table XI-2. Further comparison of two long-term studies

	Study	
Feature	**Lington et al. (1997)**	**Moore (1998)**
Levels of DINP in Diet	0.03, 0.3 and 0.6%	0.05, 0.15, 0.6 and 1.2% (Reversibility group, 1.2%)
Estimated doses received (mg $kg^{-1}d^{-1}$)	Males: 15, 152, 307 Females: 18, 184, 375	Males: 29, 88, 359, 733 Females: 36, 109, 442, 885
Survival to termination	>60% in all groups	Males: 54-78% (decreased at 1.2%) Females: 66-80%
Incidence of mononuclear cell leukemia in controls	Males: 41% Females: 27%	Males: 34% Females: 25%
Incidence of spongiosis hepatis in controls	Males: 27.2% Females: 4.9%	Males: 10.9% Females: 0%

The occurrence of spongiosis hepatis in the two studies is further compared in Table XI–3 and Figure XI-1. It is clear that a more pronounced dose response for this effect was observed in males in the study by Lington et al. (1997). The no-observed-adverse-effect level (NOAEL) was 15 mg $kg^{-1}d^{-1}$ in this study, and 88 mg $kg^{-1}d^{-1}$ in the Moore (1998) study. It is not clear which study is more appropriate for deriving an acceptable daily intake (ADI) value. The U.S. Consumer Product Safety Commission (CPSC) previously used the more sensitive Lington et al. study (CPSC, 1998; Lee, 1998). This is consistent with CPSC (1992) chronic hazard guidelines. Others have argued that the Moore study, which exhibits the less sensitive dose-response, should be used because it includes two dose levels between the NOAEL and the lowest-observed-adverse-effect level (LOAEL) in the Lington et al. study (EPL, 1999; Wilkinson and Lamb, 1999).

The Chemical Manufacturers Association (CMA), currently known as the American Chemistry Council (ACC), convened a pathology working group (PWG) to review histological slides from both studies (CMA, 2000). The PWG attributed disparity between the two studies to differences in methodology, particularly, the number of liver sections examined in each study (EPL, 1999).

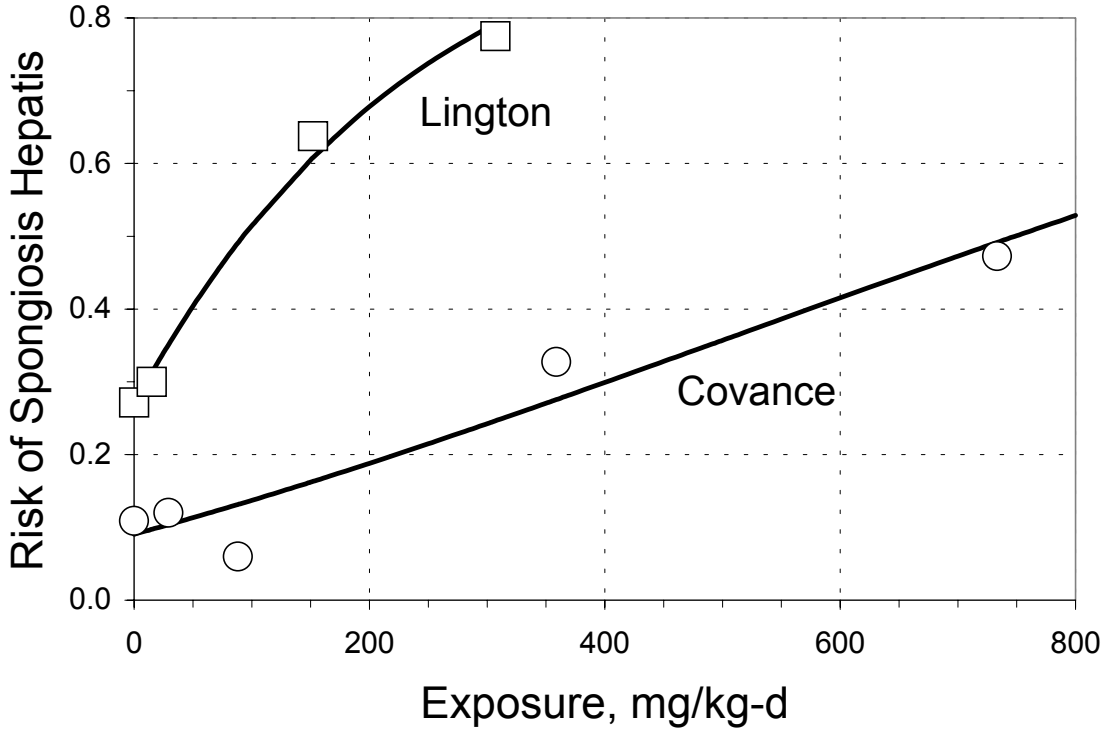

Figure XI-1. Risk of spongiosis hepatis in male rats fed diisononyl phthalate (DINP) for two years: (squares) Lington et al. (1997); (circles) Moore (1998); (curves) multistage-polynomial models fit to each data set using nonlinear least-squares (EPA, 2000). Incidence data at terminal sacrifice are as revised by the pathology working group (EPL, 1999). Adapted from Babich and Greene (2000).

The number of liver sections routinely examined was not specified in the methods sections of either study. According to the PWG, Lington et al. routinely prepared sections from each liver lobe plus gross lesions, resulting in 4 to 5 sections per liver. In contrast, Covance Laboratories (Moore, 1998) routinely reviewed only one section from each liver plus gross lesions. Because the spongiosis hepatis was generally a microscopic lesion, Lington et al. had a higher probability of finding a lesion if one existed. The difference in methodologies used complicates any comparison of the two studies. However, the CHAP agrees with the PWG's explanation that the apparent difference between the results obtained in the two studies is readily understood to be a simple consequence of the different numbers of slides examined in each study. The larger numbers of animals used in the Lington et al. study potentially contribute as well, but to a lesser extent.

Table XI-3. Incidence of spongiosis hepatis at terminal sacrifice in male F344 rats fed DINP) for 2 years in two chronic studies

Dose* mg $kg^{-1}d^{-1}$ (%)	N	X	Risk	Extra Risk	Relative Risk
Lington et al. (1997)					
0 (0)	81	22	0.272	0	1
15 (0.03)	80	24	0.300	0.039	1.10
152 (0.3)	80	51 **	0.638	0.502	2.35
307 (0.6)	80	62 **	0.775	0.691	2.85
Moore (1998)					
0 (0)	55	6	0.109	0	1
29 (0.05)	50	6	0.120	0.012	1.10
88 (0.15)	50	3	0.060	-0.055	0.55
359 (0.6)	55	18 **	0.327	0.245	3.00
733 (1.2)	55	26 **	0.473	0.408	4.33

* Dose = dose in feed (mg $kg^{-1}d^{-1}$), also listed as % of food weight in parentheses; N = number of animals at risk; X = number of animals with spongiosis hepatis at terminal sacrifice, as revised by the pathology working group (EPL, 1999); risk = fraction of animals with spongiosis hepatis (i.e., P = X/N); P_E = extra risk = $(P_D–P_0)/(1–P_0)$, where P_D is risk at dose D, and P_0 is risk at zero dose; RR = relative risk = P_D/P_0. Adapted from Babich and Greene (2000).

** Significantly different from the control at 0.01 level, two-tailed Fisher's exact test.

Since Moore (1998) and colleagues reviewed only 1 or 2 slides from each liver, the incidence of spongiosis hepatis was apparently reduced at all exposure levels, including among the controls. Based on analyses undertaken by CPSC staff with CHAP input (Babich and Greene, 2000), the dose-response data are reasonably consistent (Figure XI-2A) when expressed as relative risk (RR):

$$RR = P(D)/P_0 \quad (1)$$

where $P(D)$ is the probability of finding at least one lesion in a given animal at dose D, and P_0 is the probability of finding a lesion at zero dose. A reasonable fit to untransformed pooled data from Table XI-3 was obtained using the power dose-response model:

$$RR = 1 + bD^a \quad (2)$$

where *a* and *b* are estimated model parameters ($\chi^2 = 0.800$, df= 5, p = 0.977). The best estimate of the exponent *a* obtained was 0.88, which suggests a somewhat supralinear dose-response. The fit with this model was only marginally better than with a linear model (i.e., conditional on *a*=1; $\chi^2 = 0.836$, df= 5, p = 0.975). When the observation at 88 mg $kg^{-1}d^{-1}$ was ignored, another acceptable fit with an estimated *a*-value of 0.70 was obtained ($\chi^2 = 0.0838$, df= 5, p = 0.999). A plot of the data with dose scaled logarithmically, as presented by the Chemistry Council of America, formerly the Chemical Manufacturers Association, appears consistent with a sublinear dose-response (Figure XI-2B).

There is no way to ascertain what results would have been obtained had reviewed 4 rather than 1 slide per liver been reviewed in the Moore (1998) study. However, the effect of reviewing additional slides can be approximated by assuming that exactly one slide from each liver was examined in that study. The probability of finding a spongiosis hepatis lesion on a single slide conditional on dose *D* is represented by $p_1(D)$. Assuming that detected lesions are statistically independent, it follows that the likelihood, $p_4(D)$, of finding a lesion on 4 slides conditional on dose *D* is related to $p_1(D)$, and vice-versa, as follows:

$$p_1(D) = 1 - [1 - p_4(D)]^{1/4} \quad (3)$$

$$p_4(D) = 1 - [1 - p_1(D)]^4 \quad (4)$$

Thus, the Moore (1998) data may be scaled to make them roughly comparable to data from the Lington et al. (1997) study, and vice-versa (Table XI-4). The only dose level common to both studies is $D = 0$. As indicated in Table XI-4, when Eq. (4) is used to scale the Moore (1998) data to 1 slide/liver, the zero-dose incidence (6/55) is scaled to 20/55, which is not significantly different from the observed zero-dose incidence (22/81) in the Lington study (p = 0.34, 2-tailed Fisher exact test). Likewise, using Eq. (3) to scale the Lington data to 1 slide/liver, the scaled vs. observed (Moore 1998) incidences at zero dose (6/81 vs. 6/55) again do not differ significantly (p = 0.70, 2-tailed Fisher exact test).

Benchmark Dose Estimates. The benchmark dose (BMD) may used as an alternative to the NOAEL in setting ADI values (Crump, 1984). The principle advantage of using the BMD is that it is less sensitive than a NOAEL to the selection of experimental doses and numbers of animals per dose group. Spongiosis hepatis data were fit using the Environmental Protection Agency's (EPA, 2000) benchmark dose software to polynomial (multistage) and lognormal (log probit) dose-response models, defined as:

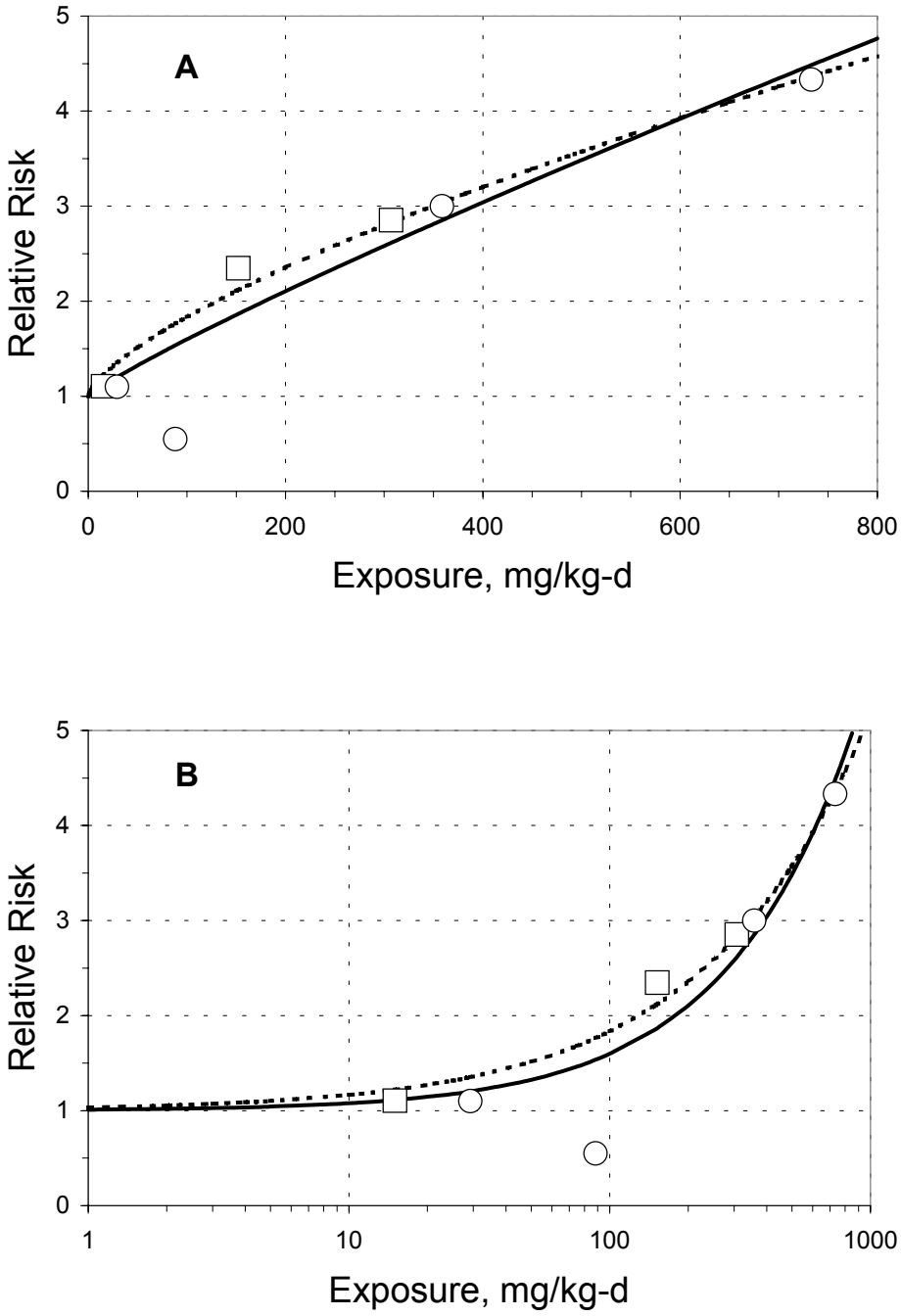

Figure XI-2. Relative risk of spongiosis hepatis in male rats fed diisononyl phthalate (DINP) for two years: (squares) Lington et al. (1997); (circles) Moore (1998). Multistage-polynomial models were fit to pooled data (solid curves) and to pooled data sans the Moore (1998) observation at 88 mg $kg^{-1}d^{-1}$ (dashed curves), using a (**A**) linear or (**B**) logarithmic exposure scale, by nonlinear least squares (EPA, 2000). Adapted from Babich and Greene (2000).

Table XI-4. Incidence of spongiosis hepatis in male rats based on pooled data from Lington et al. (1997) and Moore (1998) studies under alternative pooling assumptions

Dose, D mg kg^{-1}d^{-1}	Study	N[a]	Observed with lesion[a]		Predicted number under different pooling assumptions[a]			
					1 Slide/liver		4 Slides/liver	
			X	$p(D)$	X_1	$p_1(D)$	X_4	$p_4(D)$
0	Lington	81	22	0.272	6[b]	0.074	22[c]	0.272
0	Moore	55	6	0.109	6[b]	0.109	20[c]	0.364
15	Lington	80	24	0.300	6	0.075	24	0.300
29	Moore	50	6	0.120	6	0.120	20	0.400
88	Moore	50	3	0.060	3	0.060	10	0.200
152	Lington	80	51	0.638	17	0.213	51	0.638
307	Lington	80	62	0.775	24	0.300	62	0.775
359	Moore	55	18	0.327	18	0.327	43	0.782
733	Moore	55	26	0.473	26	0.473	50	0.909

[a]N = number of animals at risk; X = observed number of animals with spongiosis hepatis at terminal sacrifice; $p(D)$ = X/N = observed fraction of animals with spongiosis hepatis at dose D; X_1 = number and $p_1(D)$ = fraction of animals with spongiosis hepatis using observed Covance (1998) 1-slide/liver data and using Eq. (3) (see text) to scale Lington et al. (1997) data to 1 slide/liver; X_4 = number and $p_4(D)$ = fraction of animals with spongiosis hepatis using observed Lington et al. (1997) 4-slides/liver data and using Eq. (4) (see text) to scale Moore (1998) data to 4 slides/liver. Adapted from Babich and Greene (2000).

[b]These incidences are not significantly different by 2-tailed Fisher's exact test (p = 0.70).

[c]These incidences are not significantly different by 2-tailed Fisher's exact test (p = 0.34).

With the Moore (1998) data scaled to 4 slides/liver, the two dose-response relations appear similar (Figure XI-3A). Dose response models were fit to the pooled data. While marginal fits ($p \leq 0.012$) were obtained using the lognormal and polynomial models described below, good fits were obtained when the observation at 88 mg kg^{-1}d^{-1} was omitted ($p \geq 0.64$; see Table XI-5). The improvement in fit obtained by omitting that observation is statistically significant for the lognormal model (p = 0.026, by F-test).

After Lington data are scaled to 1 slide per liver, the two dose-response relations also become similar (Figure XI-3B), and are adequately fit by both models considered, whether or not the observation at 88 mg kg^{-1}d^{-1} was included ($p \geq 0.64$; see Table XI-5).

$$p(D) = p_0 + (1 - p_0)(1 - \exp[-\sum_{i=1}^{n} q_i D^i]), \quad \text{and} \tag{5}$$

$$p(D) = p_0 + (1 - p_0)\ \phi(a + b \log D), \tag{6}$$

respectively, where $p(D)$ is the probability of lesion at dose D to any ith dose group, a, b, and p_0 are parameters to be estimated, and ϕ is the cumulative standard normal probability distribution function.

Marginally better fits were obtained with the lognormal model than with the polynomial (Table XI-5). In previous work with the original Lington et al. (1997) and Moore (1998) data sets (prior to the reevaluation of the PWG), the lognormal model provided somewhat better fits in comparison to the polynomial, logistic, Weibull, and one-hit models (data not shown). For most data sets, the polynomial model required only a first order "potency" coefficient (q_1) with maximum likelihood estimates (MLEs) of higher order coefficients generally being zero. With all models, better fits were obtained with the Lington et al. data, due likely to the observation at 88 mg $kg^{-1}d^{-1}$ in the Moore data set, representing a response below the background rate. Fits obtained to the Moore data improved substantially when the observation at 88 mg $kg^{-1}d^{-1}$ was omitted (Table XI-5).

The polynomial model tended to estimate somewhat lower benchmark doses than the lognormal model. For example, using the original Moore (1998) data, the dose (D_{10}) at which extra risk is 0.1 was estimated to be 188 mg $kg^{-1}d^{-1}$ when fit by a polynomial model, compared to 215 mg $kg^{-1}d^{-1}$ when fit by a lognormal model. Lower benchmark doses were also obtained using the Lington et al. data compared to using the Moore data. For example, the MLE-D_{10} values were 26 vs. 188 mg $kg^{-1}d^{-1}$ using the Lington vs. Moore studies, when fit by polynomial models (Table XI-5). These D_{10} values are roughly double the corresponding NOAELs (15 vs. 88 mg $kg^{-1}d^{-1}$, respectively). The D_{05} values (12 vs. 98 mg $kg^{-1}d^{-1}$, respectively) are more comparable to the corresponding NOAELs.

Using pooled data with Moore data scaled to 4 slides/liver, the D_{10} value obtained with the polynomial model (33 mg $kg^{-1}d^{-1}$) was closer to the D_{10} for the Lington et al. study (26 mg $kg^{-1}d^{-1}$) than that of the Moore study (188 mg $kg^{-1}d^{-1}$). When the observation at 88 mg $kg^{-1}d^{-1}$ was omitted, the estimated D_{10} remained virtually unchanged (33 vs. 31 mg $kg^{-1}d^{-1}$) while the goodness of fit improved ($p = 0.0075$ for the full data set vs. $p = 0.64$ without the 88 mg $kg^{-1}d^{-1}$ data). Using pooled data in which Lington data were scaled to 1 slide/liver, the D_{10} estimate obtained (130 mg $kg^{-1}d^{-1}$) with a polynomial model more closely resembled that estimated from the Moore study than that from the Lington study (188 vs. 26 mg $kg^{-1}d^{-1}$, respectively). This polynomial fit was significantly better to data scaled to 1 slide/liver rather than to 4 slides/liver ($p = 0.71$ vs. 0.0075, respectively; $p=0.039$ by F-test for improved fit using polynomial model). However, all lognormal fits obtained to pooled data were statistically adequate ($p \geq 0.67$) regardless of pooling approach used (1 vs. 4 slides) or retention/omission of the Moore 88-mg $kg^{-1}d^{-1}$ data (see Table XI-5).

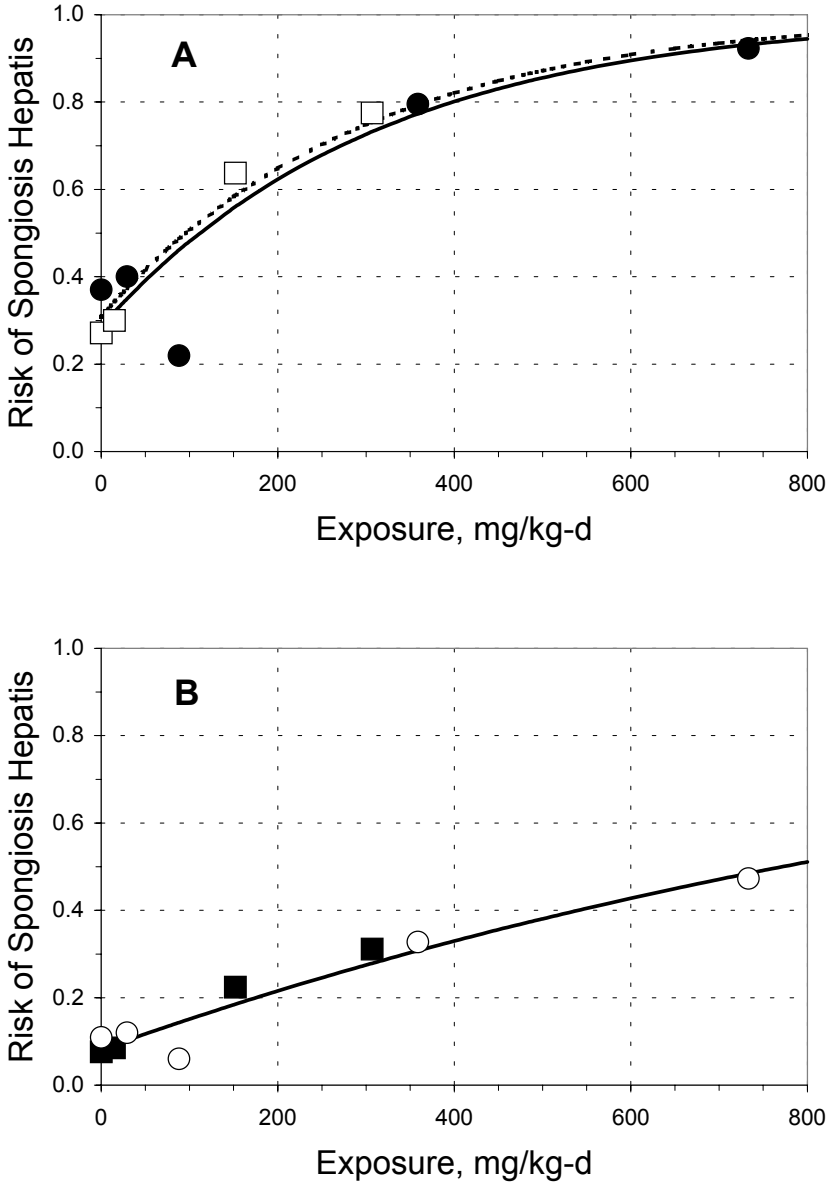

Figure XI-3. Risk of spongiosis hepatis using data from: Lington et al. (1997) (open squares), Lington et al. (1997) scaled to 1 slide/liver using Eq. (3) (solid squares), Moore (1998) (open circles), and Moore (1998) scaled to 4 slides/liver using Eq. (4) (solid circles). The pooled data (solid curves) and the pooled data sans the Moore (1998) observation at 88 mg $kg^{-1}d^{-1}$ (dashed curves), were fit to multistage-polynomial models using nonlinear least-squares (EPA, 2000). Data shown correspond to an assumption of (**A**) 4 slides per liver or (**B**) 1 slide per liver. Adapted from Babich and Greene (2000).

Table XI-5. Benchmark dose estimates for the risk of spongiosis hepatis in male rats[a]

Study	Outlier	NOAEL mg kg^{-1}d^{-1}	LOAEL mg kg^{-1}d^{-1}	Model	p-value	D_{10} (mg kg^{-1}d^{-1})		D_{05} (mg kg^{-1}d^{-1})	
						MLE	LCL	MLE	LCL
L	Include	15	152	Poly	0.78	26	20	12	10
				Log	0.92	26	7	16	3
M	Include	88	359	Poly	0.18	188	107	98	52
				Log	0.31	216	105	146	54
M	Omit	29	359	Poly	0.97	142	103	69	50
				Log	0.98	142	23	80	6
LM4	Include	88	152	Poly	0.0075	33	28	16	13
				Log	0.012	70	41	50	26
LM4	Omit	29	152	Poly	0.64	31	26	15	13
				Log	0.85	29	12	18	6
LM1	Include	88	152	Poly	0.71	130	102	—	—
				Log	0.67	145	80	—	—
LM1	Omit	29	152	Poly	0.96	128	100	—	—
				Log	0.96	111	48	—	—

[a] L = Lington et al. (1997); M = Moore (1998); LM1 = L+M data with Lington et al. (1997) data scaled to 1 liver/slide using Eq. 3 (see text); LM4 = L+M data with Moore (1998) data scaled to 4 livers/slide using Eq. 4 (see text). "Outlier" indicates either inclusion or omission of Moore 88-mg kg^{-1}d^{-1} data; Model denotes dose response model (Poly = polynomial Eq. 5, Log = lognormal Eq. 6). P = probability that deviations of observations from the model are due to chance, by chi-square test; D_{10} = estimated dose at which 10% of animals are affected; D_{05} = estimated dose at which 5% of animals are affected; MLE = maximum likelihood estimate; LCL = 95% lower confidence limit. Benchmark dose estimates were made with EPA benchmark dose software (EPA, 2000).

The two dose response curves for spongiosis hepatis differ primarily because Lington et al. (1997) reviewed 4 to 5 slides/liver, whereas Covance Laboratories (Moore, 1998) reviewed only 1 or 2 slides. Lington et al. thus had a roughly 4-fold greater chance of observing this focal lesion if present. The difference in methodology makes it difficult to compare the two studies. It is unknown whether 88 mg $kg^{-1}d^{-1}$ would have remained a NOAEL if Moore and colleagues had reviewed additional slides from each liver. Therefore, a robust approach to interpreting the available data involves normalizing the data from both studies to determine the consistency the two data sets bearing on the same toxic endpoint in male F344 rats, as described above.

Reproductive Toxicity

As summarized in Section VII, prenatal developmental toxicity has been evaluated in two studies in the rat, one by Hellwig et al. (1997), the other by Waterman et al. (1999). In the Hellwig et al. study, effects were seen only at the highest dose (1000 mg $kg^{-1}d^{-1}$) and consisted of kidney and skeletal-system alterations. A NOAEL of 200 and a LOAEL of 1,000 mg $kg^{-1}d^{-1}$ were identified for maternal and developmental effects. The Waterman et al. (1999) study used more animals and was more complete. Based on the results presented in that manuscript, the Phthalate Expert Panel convened by the Center for the Evaluation of Risks to Human Reproduction suggested to the study sponsor that additional statistical analyses using fetal incidence data be performed to evaluate the significance of skeletal variations and renal pelvis dilation. Based on this analysis, a LOAEL and NOAEL of 500 and 100 mg $kg^{-1}d^{-1}$, respectively, were determined for skeletal abnormalities (NTP/CERHR, 2000). The sponsor also calculated benchmark doses for the rudimentary lumbar rib variant, a sensitive skeletal variation endpoint, with a 5% excess risk level (BMD_{05}) of 193 mg $kg^{-1}d^{-1}$ and 95% lower confidence interval estimated by the bootstrap approach of 162 mg $kg^{-1}d^{-1}$. A two-generation study (Waterman et al., 2000) suggested an adverse effect on weight gain in pups during the perinatal and pre-weaning period, leading to a LOAEL of 250 mg $kg^{-1}d^{-1}$.

Reproductive toxicity was evaluated by Waterman et al. (2000) in standard one- and two-generation studies in the rat. Because no reproductive toxicity was observed, the NOAEL for reproductive function was 500 mg $kg^{-1}d^{-1}$, the highest exposure level evaluated. However, a single-dose study that examined more sensitive reproductive-toxicity endpoints found that oral DINP exposures of rat dams at 750 mg $kg^{-1}d^{-1}$ during critical stages of male reproductive tract development (gestational day 14 to postnatal day 3) produced a significant increase in reproductive-tract malformations (Gray et al., 2000). Because this study involved only a single dose, the dose-response for DINP-induced reproductive-tract malformations in male rats is currently unknown. This study noted that, in common with a number of other phthalate diesters (and/or their metabolites), DINP has anti-androgenic activity (Gray et al., 2000).

Adjustment/uncertainty factors

For non-carcinogenic toxic endpoints, a combined uncertainty factor of 100 is traditionally applied to the NOAEL to calculate an acceptable exposure for humans. This

value is arrived at by multiplying a factor of 10 to account for sensitivity differences between humans and experimental animals, and another factor of 10 to account for variability within the human target population. However, additional factors of 3 to 10 are also used as applicable to account for: extrapolating chronic toxicity from subchronic toxicity data, hypersensitivity of the exposed population, and/or special data gaps (Dourson et al., 1996; EPA, 1998).

To account for the potential increased sensitivity of children, application of an extra uncertainty factor of 10 for managing risks from pesticides in the dietary exposures has been proposed by the Committee on Pesticides in the Diets of Infants and Children of the National Research Council (NRC, 1992). This proposal reflects concerns regarding age-related differences in toxicity, deficiencies in current testing methods for juvenile or developing animals, and irreversibility in developing organ systems. Anatomical, biochemical and physiological changes occur during development, infancy, childhood, and adolescence that can substantially affect chemical absorption, distribution, metabolism and elimination. For example, there may be developmental periods (i.e., windows of vulnerability) when endocrine, reproductive, immune, visual, or nervous systems are particularly sensitive to certain chemicals.

The NRC Committee evaluated the extent and the health-related consequences of exposure of infants and children to pesticides, and noted the paucity of data on which to base policy (NRC, 1992, p. 4). Available data on the lethality of pesticides in young vs. adult rodents were examined. In one large data set, greater susceptibility in newborn compared to adult rodents was noted for 85% of 260 pesticides; however, because newborn rodents are less mature at birth than humans, the Committee noted that such pronounced differences would not be anticipated for humans (NRC, 1992, p. 51). In another study reviewed by the Committee, 14 to 16-day old rats were intermediate between newborn and adult rats. Only limited information was available on the therapeutic efficacy and toxicity of drugs in pediatric populations. The most complete human data set was on subacute toxicity measures (maximally tolerated doses) of cancer chemotherapeutic agents. They indicate a slightly higher tolerance among children compared to adults for the 17 mostly direct-acting compounds tabulated, though differences between age groups were ≤2-fold for all but one case, and overall were 30% (geometric mean) different. The Committee also reported several examples of significantly greater susceptibility to pesticides and pharmaceuticals in the young compared to adults, and vice versa. Several of the observed differences seen in data sets and case examples were discussed in terms of age differences in metabolism, renal clearance, and half-life. Susceptibility to acute pesticide toxicity was noted to be a function of age, species, and chemical. The Committee cautioned that the damage from acute doses of chemicals high enough to cause death may operate by mechanisms that are quite different from those that produce effects from chronic exposures at lower levels (NRC, 1992, p. 52).

The Committee concluded that “children may be more sensitive or less sensitive” and there is no simple way to predict “the sensitivity to compounds in infants and children from data derived entirely from adult humans or from toxicity testing in adult or

adolescent animals" (NRC, 1992, p. 3). The Committee noted that the most pronounced difference from adults in susceptibility would be expected in newborns and infants. It further concluded that current toxicity testing protocols do not adequately address early in life exposure and that little work has been done to identify effects that develop after a long latency period (NRC, 1992, p. 4). It emphasized that compared to late-in-life exposures, exposures early in life can lead to a greater risk of chronic effects (NRC, 1992, p. 7).

Changes in current regulatory practice were recommended (NRC 1992, p. 7). With respect to uncertainty factors, the Committee noted that although the uncertainty factor used to address variation within the human population "generally provides adequate protection for infants and children, this population subgroup may be uniquely susceptible to exposures at particularly sensitive stages of development." It recommended that "an uncertainty factor up to the 10-fold factor traditionally used by EPA and FDA for fetal developmental toxicity, should also be considered when there is evidence of postnatal developmental toxicity, and when data from toxicity relative to children are incomplete. …this is not a new, additional uncertainty factor but, rather, an extended application of an uncertainty factor now routinely used by agencies for a narrower purpose" (NRC, 1992, p. 9). Finally, "in the absence of data to the contrary, there should be a presumption of greater toxicity to infants and children. To validate this presumption, the sensitivity of mature and immature individuals should be studied systematically to expand the current limited data base on relative sensitivity" (NRC, 1992, p. 9-10). The limited available data do not establish a scientific rationale for application of an extra 10-fold factor to account for enhanced sensitivity at younger ages (Bruckner, 2000; Renwick et al., 2000), but the Committee apparently saw enough evidence to presume that generation and systematic review of such data would.

The relevance of the results of the long-term carcinogenicity bioassay with post-weaning animals to the exposure of babies and young children to DINP for a fraction of their lifetime can be questioned. In the one and two-generation reproduction studies in rats cited above, nursing offspring were exposed to DINP in the nursing mothers' milk as well as the mothers' diets during the late lactation period. Some offspring received diets containing DINP up until attainment of sexual maturity and subsequent mating to produce a second generation. While results of these reproductive/developmental studies did not show any qualitative or quantitative differences in response to DINP, they did not address chronic systemic effects like spongiosis hepatis.

With regard to potential exposure of children to DINP, the safety database has gaps that are possibly important. The long-term chronic studies in rats and mice started with animals that were proportionately older than the population of children potentially exposed to DINP from its use in toys and teethers. Even though young rats were exposed to DINP directly from ingesting their dam's diet during the latter part of the lactation period, we have no information about the long-term effects of such an exposure. Moreover the application of pharmacokinetic data derived from older animals suffers also from a failure to exactly model the human situation in infants and children. The possible

influence of a diet high in lipids as in nursing and milk-fed infants, and lack of dentition in the oral cavity, are examples of differences peculiar to human exposure scenarios.

There are, however, certain features of the human exposure scenario that may mitigate potential harmful effects from exposure to DINP. For example, exposure from toys and teethers is limited to a brief period in the lifetime. For reversible effects, such as hepatomegaly, peroxisome proliferation, and possibly spongiosis hepatis, this may reduce potential danger. Furthermore, there are no experimental data indicating that DINP produces more toxic effects in young vs. older animals. In summary, there is no direct scientific evidence for DINP to support application of safety factors greater than the 100 value indicated above. Still, given the limited data for evaluation, concerns remain for both PPARα mediated effects and those, such as antiandrogenic effects (e.g., testicular) and spongiosis hepatis, presumed not associated with PPARα activity. Thus the CHAP recommends experimental investigation to provide a better foundation for regulatory decisions on this and other phthalates in consumer products.

B. DINP Risk Assessment for Carcinogenic Endpoints

The CHAP concludes that, based on information reviewed in Chapter X of this report, DINP causes liver cancer in rodents by a PPARα-mediated mechanism, that is pronounced in rodents and believed not readily induced in humans, especially at doses resulting from current use of consumer products. The findings of mononuclear cell leukemia and renal tubular carcinoma in the rodent bioassays for DINP are of questionable relevance to humans.

The CHAP therefore concludes that humans will not receive DINP doses from current uses of DINP-containing consumer products that are associated with a significant increase in cancer risk.

C. Derivation of an Acceptible Daily Intake

The selection of a study to derive acceptable daily intake (ADI) is based on the most sensitive species and more sensitive gender when there are differences in response. Based on DINP toxicity data reviewed above, the most sensitive toxicity endpoint that clearly has been established for DINP is spongiosis hepatis in male F344 rats. An ADI for DINP is derived from the corresponding dose response data by fitting a mathematical model to estimate a Benchmark Dose (BD), then dividing by an uncertainty/adjustment factor.

All lognormal fits to pooled data were adequate ($p \geq 0.67$) regardless of pooling approach used (1 vs. 4 slides) and whether or not the outlier in the Moore (1998) data set was retained or omitted (i.e., the 88-mg $kg^{-1}d^{-1}$ data point). As expected, analyses based on 4 slides per liver or with the outlier removed yielded lower BD estimates than those based on one slide per liver or including the outlier. The MLE BD_{05} of 15 mg $kg^{-1}d^{-1}$ (Table

XI-5) based on the pooled Lington et al. (1997) and Moore (1998) data sets, with outlier omitted, is comparable to the MLE BD_{05} of 12 mg $kg^{-1}d^{-1}$ from the more sensitive Lington et al. (1997) study. The ADI corresponding to the latter value is 0.120 mg $kg^{-1}d^{-1}$, based on the application of a 100-fold combined uncertainty/adjustment factor. A lower ADI would have been obtained had an additional uncertainty/adjustment factor been employed to address uncertainty regarding differences between adults and children, residual risk at the benchmark dose (5%), or the use of an MLE rather than lower bound benchmark dose.

D. Comparison of the Acceptable Daily Intake with Exposure Estimates

Background DAP exposures have been considered in previous assessments of potential risk from DAPs in children's products, under the assumption that toxicological effects of DAPs are additive (EU, 1998; RIVM, 1998). DAPs differ in their toxic endpoints and potencies. For example, some, but not all DAPs are carcinogenic, teratogenic, and/or reproductive toxicants in animals. A common feature of DAPs is their ability to induce liver toxicity (ATSDR, 1990; ATSDR, 1993; NTP, 1995; NTP, 1997a,b,c; reviewed in EU, 1998). Given the structural similarity of DAPs, it is likely that these liver effects are due to similar mechanisms. However, there are no data demonstrating additivity of DAP-induced toxic effects. Even if they act through a common mechanism, DAP effects are not necessarily additive. Still, for low dose exposures, the assumption of additivity is a generally accepted conservative approach to addressing this source of uncertainty, as well as one that has theoretical support in the case that damage occurs by statistically independent increments (NRC, 1994).

DINP exposure from children's products are in addition to other DAP exposures, including possible oral and dermal DAP exposures from a variety of consumer products, as discussed in Section IV and the Appendix. To quantitatively assess the impact of background exposures requires: (a) a method of adjusting for potency differences among different DAPs, and (b) an estimate of background DAP exposure in infants. There are several different ways to adjust for the potency of different DAPs, including use of toxic equivalency factors, such as those used for dioxin congeners, or a fractional equivalent dose method, or by assuming that all DAPs are equipotent. If the relative concentrations of different DAPs are unknown, assuming potencies equal to the most potent DAP identified is a reasonably cautious approach.

Estimates of DAP exposures in the U.S., that include reliable estimates for the important possible sources (see e.g., the Appendix and Section IV), are not available. Estimates of background exposure in the general population taken from the literature range from 1 to 23 μg $kg^{-1}d^{-1}$ (see Section IV). The approximate geometric mean of this range (~5 μg $kg^{-1}d^{-1}$) is <5% percent of the ADI value for DINP (120 μg $kg^{-1}d^{-1}$) and is a negligible fraction of reasonable upper-bound DINP exposures estimated to be associated with consumer products listed in Table IV-7 (Section IV). On the other hand, the study of Blount et al. (2000) measuring urinary levels of seven phthalates in adults in the U.S. suggests a fraction of the adult population may have exposures at or above 100 μg $kg^{-1}d^{-1}$.

Because of the difficulty obtaining reliable estimates of phthalate exposure for the population of interest (infants and toddlers) and uncertainties on how exposure estimates should be combined for comparison with the ADI, further explicit consideration of environmental background DAP exposures is not undertaken in this report. Likewise, this report does not explicitly consider possible effects of potential joint exposures to DINP and other phthalate diesters that may involve dermal uptake from specific consumer products worn, mouthed or otherwise in prolonged contact with skin (see Section IV.C). Fundamental uncertainties currently prevent reliable quantitative assessment of such dermal exposures under realistic exposure conditions (see Appendix A).

One of the two estimates of plausible upper-bound DINP exposures listed in (Section IV, Table IV-7) is greater than the ADI of 0.12 mg $kg^{-1}d^{-1}$ recommended for DINP, namely, the estimate of 0.28 mg $kg^{-1}d^{-1}$ listed for ingested DINP among children 0-18 months old who mouth PVC plastic toys that contain DINP for 3 hours/day. This implies that there may be a DINP risk for any young children who routinely mouth DINP-plasticized toys for 75 minutes/day or more.

As noted in Section IV.C, some amount of percutaneous DINP exposure is expected from dermal or oral contact with bedding, clothing, or footwear made of PVC products that may be plasticized using DINP. The extent to which such products exist is not known. Data from *in vitro* and *in vivo* studies involving dermal exposures to neat DINP are consistent with the hypothesis that all such potential dermal exposures to DINP are negligible, whereas current theoretical models predict non-negligible DINP uptake by skin or oral mucosa in exposures that involve contact with dilute aqueous DINP. In the absence of detailed data on the prevalence of DINP in consumer products that are in sustained dermal contact, and in view of the present fundamental uncertainty in the magnitude of possible dermal DINP uptake (see Appendix), current estimates of potential dermal exposure from such products remain speculative. If theoretical models turn out to predict reasonably well, a substantial number of children will be exposed above the recommended ADI. In view of the range of potential dermal exposures to DINP that can be estimated for a few types of consumer products (see Appendix), additional experimental data should be developed. Such experimentation could substantially reduce current uncertainties concerning the magnitude of potential risks associated with dermal exposures to DINP and other phthalate esters from consumer products.

E. Conclusions

Humans will not receive DINP doses from current uses of DINP-containing consumer products that are associated with a significant increase in cancer risk. This conclusion is based on DINP-exposure studies that demonstrated a lack of DINP-induced tumors or peroxisomal induction (deemed requisite for DINP-induction of tumors) in primates exposed chronically to DINP doses as high as 2,500 mg $kg^{-1}d^{-1}$, as well as in view of the lack of human epidemiological data indicating any liver cancer risk due to chronic clinical exposure to drugs that are far more potent at inducing peroxixomes in rodent than

are DINP and related plasticizers such as DEHP. Consequently, the CHAP concludes that linear extrapolation of cancer risk for DINP is not scientifically warranted. The positive mouse liver-tumor endpoint should thus be treated as a classical form of chronic toxicity for risk assessment purposes, similar to reproductive/developmental toxicity and spongiosis hepatis, the latter being the most sensitive endpoint identified as discussed above.

The ADI for DINP is based on the most sensitive toxicity endpoint, species and gender, in this case spongiosis hepatis in male F344 rats. It is derived by fitting a mathematical model to the corresponding dose response data to estimate a BD_{05} of 12 mg $kg^{-1}d^{-1}$, then dividing by an uncertainty/adjustment factor of 100. For a subset of children 0-18 months old who mouth PVC plastic toys that contain DINP for 75 minutes/day or more, exposures are expected to exceed the ADI. For the majority of children, the exposure to DINP from DINP containing toys would be expected to pose a minimal to non-existent risk of injury.

The exposure estimates addressed oral exposures only. Dermal exposure is expected from products plasticized with DINP in prolonged contact with external skin or oral mucosa. Estimates of potential dermal exposure from such products remain speculative. If theoretical models are reasonable predictors, a substantial number of children are exposed above the ADI. If instead, experiments using neat DINP are reasonable predictors dermal exposure is negligible. The CHAP recommends experiments be undertaken to reduce this important source of uncertainty in the risk characterization.

The CHAP identified a number of additional uncertainties associated with the determinations of exposure, hazard, and dose response. Those associated with exposure include:

- lack of knowledge about what portion of toys contain DINP
- lack of knowledge about what other consumer products contain DINP
- lack of knowledge about how much DINP migrates out of toys and other consumer products
- uncertainties about how much time each day a child spends with toys and other DINP containing objects in their mouths
- lack of knowledge about how much if any DINP would be dermally absorbed

Uncertainties associated with the hazard and dose response include:

- the degree to which spongiosis hepatis in rodents is relevant to humans
  - how to extrapolate an effect from a lifetime exposure in rodents to a two-to-three year exposure in young children
  - lack of knowledge of effects of early in life exposures; there are no toxicological data for exposures corresponding to infancy and toddler years.
  - lack of knowledge of effects in non-rodents; there are no chronic studies in non-rodent mammals.

- lack of knowledge of PPARα expression and related responses in the young; there are no data in human infants and children and scant data in non-human species.
- lack of knowledge on mechanisms by which PPARα induces rodent liver tumors

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APPENDIX A

Potential Dermal Exposures to DINP from Consumer Products

Because DINP can leach from consumer products in contact with skin and oral mucosa into sweat and saliva, the CHAP attempted to derive related estimates of dermal exposure. Unfortunately only limited data are available for estimating the rate of uptake of DINP into human skin exposed to low aqueous concentrations.

Dermal absorption of ^{14}C-DINP was studied in male Fischer 344 rats (Midwest Research Institute, 1983) in both conditioned (pre-treatment with non-labeled DINP) and non-conditioned skin. Following exposure, the dosed area was occluded. Under all conditions, the amount absorbed after 7 days ranged from 2–4% of the dose. Approximately 93–99% of the administered radioactivity was recovered at the site of application. Radioactivity in feces and gut of the exposed rats suggested some excretion via the biliary route. This is consistent with a structure-activity relationship of dermal absorption of phthalate esters with varying length of sidechains. Short-chain diesters of phthalic acid may penetrate rat skin readily, while longer chain diesters are poorly absorbed (Elsisi et al., 1989). Butyl, ethyl, methyl diesters demonstrate 6% to 24% of the administered dose absorbed through rat skin in 24 hours whereas hexyl, ethylhexyl, and isodecyl diesters demonstrate 1% or less of the administered dose dermally absorbed in 24 hours. DINP is structurally similar to the latter group and would therefore not be expected to be absorbed dermally to any significant extent. Therefore, hepatic exposure to DINP as a result of dermal contact to products containing DINP resulting in spongiosis hepatis is considered insignificant for the present risk characterization.

In *in vitro* studies comparing absorption of DEHP through human and rat skin (Scott et al., 1987), absorption through human skin was slower than through rat skin. Therefore, the *in vivo* dermal absorption rate of DINP is also expected to be slower through human than through rat skin. These studies used neat DINP. Studies of dermal uptake of DINP from dilute aqueous solutions, to investigate uptake from sweat and saliva, have not been conducted.

In an *in vivo* study of the migration of DEHP plasticizer from PVC film into rat skin, 15-cm^2 sheets of this film were applied to shaved backs of rats. The mean dermal DEHP uptake rate was small, roughly 0.24 μg cm^{-2} h^{-1} (Deisinger et al., 1998). *In vitro* diffusion-cell studies of transdermal permeability of neat DEHP have shown that permeability of phthalates through human *stratum corneum* is 2- to 4-fold less than that through rat skin (Scott et al., 1987; Barber et al. 1992), and this *in vitro* species difference is likely to be true for neat DINP as well.

The extent to which such *in vivo* rat experiments predict DINP uptake into human skin exposed to low aqueous DINP concentrations, or skin in abrasive contact with DINP-containing PVC, is not known. In contrast to shaved rat skin in dry, static contact with neat PVC, plasticizer can migrate from PVC plastic (e.g., from sandals or a mouthed baby toy) into sweat produced by human skin, or into saliva in the mouth, under moist,

dynamic, or mildly abrasive exposure conditions. Exposure to neat organic compounds can alter the barrier/transport properties of lipid in *stratum corneum*. For this reason, *in vitro* measures of the permeability of neat phthalate compounds into human and rat skin made by Scott et al. (1987) were specifically excluded by the U.S. EPA (1992) from data that were considered relevant to predicting human dermal exposure at low aqueous concentrations. Similar data reported later by Barber et al. (1992), which were not considered in the U.S. EPA (1992) dermal exposure assessment guidelines document, would likewise be disqualified if the same criteria were applied.

The capacity for percutaneous uptake of a chemical at low aqueous concentration in contact with skin is characterized by the value of that chemical's concentration-independent "effective" permeability constant, K_p^{eff} (cm h^{-1}). The unit of K_p^{eff} is more easily understood as the number of mL of aqueous solution in contact with skin that are completely cleared by percutaneous uptake of the dissolved chemical per hour of contact by each cm^2 of skin in such contact (McKone, 1993). K_p^{eff} measurements are not available for DINP or related compounds. K_p^{eff} for this compound through skin regions having typical dermal permeability may be estimated using a model recently proposed specifically for use as a method to predict dermal uptake of organic chemicals in dilute aqueous concentration in the context of environmental risk assessment (EPA, 1992; Cleek and Bunge, 1993; Bunge and Cleek, 1995). The EPA-Bunge-Cleek (EPABC) model incorporates general relationships exhibited by *in vitro* diffusion-cell measures of dermal permeability (K_p, cm h^{-1}) obtained under steady-state conditions. Such data are predicted by regression models involving physicochemical variates such as molecular weight (MW, unitless) and octanol/water partition coefficient (K_{OW}, unitless). In particular, the EPABC model (Cleek and Bunge, 1993; Bunge and Cleek, 1995) incorporates the approximate relation fit by Potts and Guy (1992) to data compiled by Flynn (1990) on 93 organic chemicals, for all of which $\log_{10}(K_{OW}) \leq 5.5$ ($r = 0.82$, $p < 10^{-16}$):

$$\log_{10}(K_p, \text{cm h}^{-1}) \approx -2.74 - 0.0061\, MW + 0.71 \log_{10}(K_{OW}) \qquad (1)$$

Because for DINP $\log_{10}K_{OW} \approx 9$ (Staples et al., 1997) and MW = 418.62, the estimated K_p value for DINP based on Eq. (1) is approximately 12 cm h^{-1}. This K_p estimate is 6 to 8 orders of magnitude larger than indicated by diffusion-cell data obtained *in vitro* using rat and human skin tissue exposed to neat DINP under steady state conditions, i.e., after the "lag time" (t^*) required for DINP to reach steady-state equilibrium in the experimentally exposed skin tissue (Scott et al., 1987; Barber et al. 1992). However, exposure durations used in these *in vitro* studies were ≤ 32 h, whereas the EPABC model predicts that t^* for DINP in *stratum corneum* is about 6.5 days (cf. Bunge and Cleek, 1995; Eqs. 4a-f in Bogen et al., 1998). This estimated lag time for DINP is substantially less than the normal (~19-day) turnover time of human *stratum corneum* (Wilhelm et al., 1990; Effendy et al., 1996). Therefore, *in vitro* diffusion-cell data available for phthalate diesters may not accurately reflect permeability expected under relatively chronic exposure conditions.

Under conditions involving dermal exposures to aqueous DINP that are sustained (relative to t^*), the EPABC model predicts that K_p^{eff} for DINP is (substantially) greater than approximately $K_p/100$, i.e., that $K_p^{eff} > 0.12$ cm h^{-1} (cf. Bunge and Cleek, 1995; Eqs. 4a-f in Bogen et al., 1998). The EPABC model was specifically intended to be used to estimate dermal permeability of low aqueous concentrations of phthalate diester plasticizers, K_p estimates for three of which (including DEHP) are listed in the EPA (1992) report. Nevertheless, the applicability of Eq. (1) to estimate K_p for DINP, and the corresponding EPABC estimate of K_p^{eff}, is clearly questionable insofar as they are based on a regression fit to *in vitro* diffusion-cell data for aqueous solutions of chemicals that all have K_{OW} values ≤5.5, i.e., many orders of magnitude less than the value of K_{OW} for DINP.

An alternative to the EPABC model is the following regression model,

$$\log_{10} K_p^{eff} = -0.812 - 0.0104MW + 0.616 \log_{10} K_{OW} \quad (R^2 = 0.98, p = 3\times10^{-6}) \qquad (2)$$

This does not involve t^* and was based on all (nine) available measures of K_p^{eff} made for organic chemicals in aqueous solution based on *in-vivo* experiments, most of which involved human subjects (Bogen, 1994). Bogen (1994) proposed this model to address the fact that the EPABC model substantially underpredicts all nine of the K_p^{eff} estimates obtained from *in vivo* studies involving dermal exposures to dilute aqueous solutions of organic chemicals. Based on Eq. (2), the estimated K_p^{eff} value for DINP is 2.4 cm h^{-1}.

The K_p^{eff} estimate for DINP based on the Bogen model (Eq. 2) is relatively high compared to those of other lipophilic environmental contaminants, whereas the minimum value estimated using the EPABC model (Eq. 1) is within the range of many lipophilic organic contaminants (EPA, 1992). For comparison, K_p^{eff} for dilute aqueous trichloroethylene was estimated to be 0.2 to 0.3 cm h^{-1}, both by *in vivo* methods applied to hairless guinea pigs, and by direct measures made using accelerator mass spectrometry (AMS) applied to human surgical skin tissue exposed *in vitro* (Bogen et al., 1992, 1998).

There are no data available concerning dermal absorption of DINP specifically from the oral cavity, in contrast to external skin surfaces. As reviewed previously regarding potential DEHP uptake through the oral mucosa (CPSC, 1985), the oral cavity is lined with stratified squamous epithelium like the surface of the skin, not with the simple columnar epithelium of the type lining the gastrointestinal tract. Saliva directly in contact with the oral cavity surface maintains this surface in a moist state. Saliva and the stratified squamous epithelium perform an important barrier function against absorption via the oral cavity. However, hydration of epithelial lining tissue may increase the permeability of the oral mucosa (Squier and Johnson, 1975). There are different mechanisms by which xenobiotics penetrate epithelial tissue, including endocytosis, active transport, intercellular movement, and diffusion. Several factors are expected to

affect penetration through oral mucosa, including physicochemical properties of the compound such as its molecular size and oil/water partition coefficient (see Eqs. 1-2, and associated text and references). No direct comparisons have been made between the permeability of oral mucosa and that of other, less hydrated and less porous body areas for a range of lipophilic compounds. However, dermal uptake of lipophilic organic compounds has been observed to be roughly 5 to 40 times greater in relatively hydrated and porous dermal regions such as the forehead and scrotum compared to less hydrated and less porous dermal regions such as torso and forearm regions (Wester and Maibach, 1983).

Consequently, it is possible that substantial DINP uptake occurs via percutaneous transfer through oral mucosal tissue when, e.g., children mouth plastic items such as pacifiers or teething toys. If this does occur, then it would also occur during *in vivo* mouthing experiments undertaken to estimate DINP extraction during the mouthing of consumer products (see Section IV.C), causing those experiments to underestimate the true extent of such DINP extraction. Uncertainty regarding whether such underestimation has occurred due to human dermal uptake of DINP (and/or DINP metabolites) through the oral mucosa could be resolved experimentally by comparing DINP levels in actual or simulated saliva that is pre-spiked with a known DINP concentration and then mouthed by subjects for a specified duration. This type of experiment might most conveniently be done using accelerator mass spectrometry to measure salivary amounts of DINP that is ^{14}C-radiolabeled at an ultra-low (and thus harmless) activity level (Bogen, 1998).

As noted above, all available experimental data concerning dermal permeability of DINP (and of other phthalate diesters) are based on the application of neat compound. Moreover, Eqs. (1-2) are both based on fits to limited sets of experimental data all involving compounds having a K_{OW} many orders of magnitude less than K_{OW} for DINP. Consequently, there clearly is great uncertainty in K_p^{eff} for DINP. This uncertainty could be reduced by measuring K_p^{eff} for subchronic dermal exposure to dilute aqueous DINP *in vivo*. Alternatively, an ultrasensitive (e.g., AMS) method could easily be used to obtain a very accurate characterization of the initial kinetics of dermal DINP uptake from dilute aqueous solution. This information then could be used to estimate uptake under prolonged exposure conditions using a physiologically based compartmental model (Bogen, 1998).

At present, a range of plausible upper bounds on DINP exposure from plasticized clothing items via the dermal route (including via the oral mucosa) may be obtained using applicable flux or permeability estimates discussed above. Specifically, dermal uptake of DINP may be estimated using the effective dermal flux (F_d) estimate of 0.24 μg cm^{-2} h^{-1} obtained by Deisinger et al. (1998) for DEHP migration into rat skin during subchronic, static exposure to a DEHP-plasticized PVC plastic sheet. This approach will be referred to as the Contact-Flux (CF) method. Alternatively, dermal uptake of DINP may be estimated using what shall be referred to as the "Aqueous-Clearance" (AC) method based on either K_p^{eff} estimate (>0.12 or 2.4 cm h^{-1}) derived using Eqs. (1) or (2) as described above. Application of the AC method presumes that humid conditions lead to sustained dermal contact with a volume of perspiration <0.12 mL (or <2.4 mL) per cm^2 of

underlying skin according to the EPABC (or Bogen) model, into which DINP from plasticized clothing is continuously extracted, analogous to DINP extraction associated with mouthing of children's toys discussed above. The AC method requires that

$$C_{max} > F_d / K_p^{eff} \quad (3)$$

where K_p^{eff} is the effective permeability coefficient for DINP (>0.12 or 2.4 cm h^{-1}, based on the EPABC or Bogen models, respectively), C_{max} is the maximum possible aqueous concentration of DINP, and F_d is the effective dermal flux permitted by migration of DINP from the PVC plastic in contact with the assumed aqueous medium between plastic and skin. The 95% upper confidence bound on CPSC's estimated *in vivo* DINP extraction from children's toys (60 μg cm^{-2} h^{-1}) shall be used to estimate F_d for extracted DINP available for percutaneous uptake through oral mucosa. This F_d value is also a reasonable upper limit on possible DINP extraction by sustained contact of external skin with plasticized clothing or footwear under moist and dynamic contact conditions. Realistic dermal-contact conditions, however, are probably substantially less efficient than extraction in the extreme case of PVC clothing items being chewed for the same period of time. For purposes of the present illustration of dermal exposure assessment for DINP using the AC method, it is assumed arbitrarily that extraction efficiency relative to chewing is 10%. Hence the potential effective flux (F_d), of DINP from PVC clothing or footwear of 6 μg $cm^{-2}h^{-1}$. Application of the AC method thus involves an assumed F_d value that is 25 times greater than that using the CF method. The assumed F_d values of 6 and 60 μg $cm^{-2}h^{-1}$ for PVC contact with external skin and with oral mucosa, respectively, imply that C_{max} must be (1) >50 or >2.5 μg/mL for AC methods to predict external dermal DINP uptake based on the EPABC or Bogen model, respectively, and (2) >500 or >25 μg/mL for corresponding AC methods to predict DINP uptake through oral mucosa. Because all of these conditions are assumed for the present analysis, the AC method yields identical results using either the EPABC or the Bogen model to predict dermal DINP uptake.

Table A-1 below provides estimates of plausible upper bounds on potential dermal exposures to DINP from selected types of consumer products, using both the CF and AC methods.

Dermal Exposures to any PVC Plastic Clothing that May Contain DINP

Exposure to DINP could occur among young children or adults who wear PVC plastic clothing, including rainwear or dancing/fashion clothing, if any of these products are plasticized using DINP (see Section IV). Assuming body weights of 10 vs. 70 kg for children (19-36 months) vs. adults, and a total of 400 cm^2 of skin in contact with a DINP-plasticized pair of rainpaints and/or raincoat for 4 h d^{-1} during 30 d y^{-1}, the corresponding time-weighted average daily DINP exposures over the year estimated using the AC method would be 79 vs. 11 μg kg d^{-1} for children (19-36 months) vs. adults, respectively. Note that for these calculations, the EPABC and Bogen models were used together with an effective extraction rate of 6 μg $cm^{-2}h^{-1}$. Corresponding time-weighted average daily dermal DINP exposures would calculated using the CF method applied to the same

exposure conditions are approximately 3.2 vs. 0.45 μg kg d^{-1} for children (19-36 months) vs. adults, respectively.

Summary of Plausible Upper Bounds on Selected Dermal DINP Exposures

Estimates of potential dermal DINP exposure are summarized, together with corresponding estimates of ingestion exposure (Section IV, Table IV-7) where applicable, in Table A-1. These estimates indicate that plausible upper-bound estimates of dermal DINP exposure calculated using the CF method are negligible, e.g., in comparison with a proposed acceptable daily intake (ADI) of 0.12 mg $kg^{-1}d^{-1}$ (see Section XI). In contrast, plausible upper-bound estimates of dermal DINP exposure obtained using the AC method are $\geq$0.12 mg $kg^{-1}d^{-1}$ in the case of hypothetical DINP-plasticized pacifiers and other toys mouthed by 0-18-month-old children, and in the case of hypothetical DINP-plasticized PVC sandals worn by 10-kg children. As noted in Section IV.E, in the absence of detailed data on the prevalence of DINP in consumer products that are in sustained contact with external skin and/or oral mucosa, and in view of present fundamental uncertainty concerning the magnitude of dermal DINP uptake discussed in Section IV.C, current estimates of potential dermal exposure from such products remain speculative.

Table A-1. Combined summary of estimated plausible upper-bound DINP exposures from different consumer products, including potential (hypothetical) dermal exposures estimated using two different methods.

Age group	Object type[a]	Method[b]	Plausible upper-bound estimates of DINP exposure (μg kg^{-1} d^{-1})[a]		
			Ingestion	Dermal	Total[b]
0-18 months	Pacifier	CF	260	3.1	260
	Other toy	CF	280	1.1	280
19-36 months	Pacifier	CF	180	2.1	180
	Other toy	CF	66	0.26	66
	Rainwear	CF	–	3.2	3.2
	Sandals	CF	–	14	14
Adult	Rainwear	CF	–	0.45	0.45
	Sandals	CF	–	3.9	3.9
0-18 months	Pacifier	AC	260	210	>260 - 470
	Other toy	AC	280	150	>280 - 330
19-36 months	Pacifier	AC	180	64	>180 - 240
	Other toy	AC	66	15	>66 - 75
	Rainwear	AC	–	79	79
	Sandals	AC	–	340	340
Adult	Rainwear	AC	–	11	11
	Sandals	AC	–	98	98

[a] Illustrative dermal exposure assessments were performed for the objects listed assuming that each specified object was plasticized using DINP. Published data are not currently available allowing a quantitative characterization of DINP content in consumer products other than pacifiers and mouthed children's toys (see Section IV). The method used to estimate DINP ingested after oral extraction from pacifiers is the same as that used in Section IV for non-pacifier toys, except a 3-cm^2 pacifier surface area and a 10-hour daily exposure were assumed.

[b] CF = contact-flux method, AC = aqueous-clearance method, where CF and AC designate the method used to estimate dermal exposure (see text); "–" indicates not applicable. Estimates of total DINP exposure listed that combine ingestion and dermal uptakes assume (conservatively) that DINP extractions in the oral cavity were underestimated.

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APPENDIX B

NTP Analysis of the Covance Bioassay Data

A. F344 Rat

Table B-1. Statistical Analysis of Kidney Renal Tubule Carcinoma in Male Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	0/65 (0%)	0/55 (0%)	0/55 (0%)	0/65 (0%)	2/65 (3%)
Poly-3-Rate (b)	0/56.56	0/51.36	0/49.57	0/54.07	2/51.88
Poly-3 Percent (g)	0.0%	0.0%	0.0%	0.0%	3.9%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	0/10 (0%)
Terminal (d)	0/43 (0%)	0/40 (0%)	0/44 (0%)	0/38 (0%)	0/32 (0%)
First Incidence	--	--	--	--	660
Statistical Tests					
Life Table	p=0.13*	(e)	(e)	(e)	p=0.186
Poly 3	p=0.22	(e)	(e)	(e)	p=0.219
Poly 1.5	p=0.024	(e)	(e)	(e)	p=0.227
Poly 6	p=0.20	(e)	(e)	(e)	p=0.207
Logistic Regression	p=0.029	(e)	(e)	(e)	p=0.245
Coch-Arm/Fishers	p=0.026	(e)	(e)	(e)	p=0.248

Table B-2. Statistical Analysis of Kidney Renal Tubule Carcinoma in Female Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	0/65 (0%)	0/49 (0%)	0/50 (0%)	0/65 (0%)	0/65 (0%)
Poly-3-Rate (b)	0/53.69	0/44.98	0/47.64	0/54.52	0/52.54
Poly-3 Percent (g)	0.0%	0.0%	0.0%	0.0%	0.0%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	0/10 (0%)
Terminal (d)	0/42 (0%)	0/38 (0%)	0/40(0%)	0/39 (0%)	0/38 (0%)
First Incidence	--	--	--	--	--
Statistical Tests					
Life Table	(e)	(e)	(e)	(e)	(e)
Poly 3	(e)	(e)	(e)	(e)	(e)
Poly 1.5	(e)	(e)	(e)	(e)	(e)
Poly 6	(e)	(e)	(e)	(e)	(e)
Logistic Regression	(e)	(e)	(e)	(e)	(e)
Coch-Arm/Fishers	(e)	(e)	(e)	(e)	(e)

Table B-3. Statistical Analysis of Liver Hepatocellular Adenoma in Male Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	4/65 (6%)	3/50 (6%)	2/50 (4%)	6/65 (9%)	10/65 (15%)
Poly-3-Rate (b)	4/57.27	3/46.38	2/44.61	6/54.37	10/51.92
Poly-3 Percent (g)	7.0%	6.5%	4.5%	11.0%	19.3%
Int Sacrifice 1 (c)	1/10 (10%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	0/10 (0%)
Terminal (d)	2/43 (5%)	2/35 (6%)	1/39(3%)	4/38 (11%)	8/32 (25%)
First Incidence	549 (I)	723	718	665	639
Statistical Tests					
Life Table	p<0.001**	p=0.591	p=0.530N	p=0.310	p=0.027
Poly 3	p=0.006**	p=0.614N	p=0.457N	p=0.338	p=0.050
Poly 1.5	p=0.008**	p=0.624N	p=0.465N	p=0.352	p=0.061
Poly 6	p=0.003**	p=0.603N	p=0.442N	p=0.320	p=0.037*
Logistic Regression	p=0.003**	p=0.641N	p=0.463N	p=0.347	p=0.045*
Coch-Arm/Fishers	p=0.012*	p=0.659	p=0.471N	p=0.372	p=0.078

Table B-4. Statistical Analysis of Liver Hepatocellular Adenoma in Female Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	0/65 (0%)	1/49 (2%)	0/50 (0%)	1/65 (2%)	3/65 (5%)
Poly-3-Rate (b)	0/53.69	1/45.07	0/47.64	1/54.52	3/53.83
Poly-3 Percent (g)	0.0%	2.2.%	0.0%	1.8%	5.6%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	1/10 (10%)
Terminal (d)	0/42 (0%)	0/38 (0%)	0/40(0%)	1/39 (3%)	0/38 (0%)
First Incidence	--	704	--	728 (T)	550 (I)
Statistical Tests					
Life Table	p=0.043*	p=0.490	(e)	p=0.485	p=0.121
Poly 3	p=0.036*	p=0.465	(e)	p=0.503	p=0.120
Poly 1.5	p=0.038*	p=0.456	(e)	p=0.503	p=0.121
Poly 6	p=0.033*	p=0.476	(e)	p=0.503	p=0.119
Logistic Regression	p=0.048*	p=0.460	(e)	p=0.485	p=0.110
Coch-Arm/Fishers	p=0.041*	p=0.430	(e)	p=0.500	p=0.122

Table B-5. Statistical Analysis of Liver Hepatocellular Carcinoma in Male Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	1/65 (2%)	0/50 (0%)	0/50 (0%)	1/65 (2%)	12/65 (18%)
Poly-3-Rate (b)	1/56.56	0/46.36	0/44.57	1/54.07	12/52.09
Poly-3 Percent (g)	1.8%	0.0%	0.0%	1.9%	23.0%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	1/10 (10%)
Terminal (d)	1/43 (2%)	0/35 (0%)	0/39(0%)	1/38 (3%)	10/32 (31%)
First Incidence	728(T)	--	--	728(T)	549 (I)
Statistical Tests					
Life Table	p<0.001**	p=0.541N	p=0.519N	p=0.310	p=0.734
Poly 3	p<0.001**	p=0.540N	p=0.548N	p=0.338	p=0.751
Poly 1.5	p<0.001**	p=0.545N	p=0.551N	p=0.352	p=0.755
Poly 6	p<0.001**	p=0.534N	p=0.540N	p=0.320	p=0.745
Logistic Regression	p<0.001**	(e)	(e)	p=0.347	p=0.734
Coch-Arm/Fishers	p<0.001**	p=0.565N	p=0.565N	p=0.372	p=0.752N

Table B-6. Statistical Analysis of Liver Hepatocellular Carcinoma in Female Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	1/65 (2%)	0/49 (0%)	0/50 (0%)	1/65 (2%)	5/65 (8%)
Poly-3-Rate (b)	1/53.69	0/44.98	0/47.64	1/54.52	5/52.55
Poly-3 Percent (g)	1.9%	0.0%	0.0%	1.8%	9.5%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	0/10 (0%)
Terminal (d)	1/42 (2%)	0/38 (0%)	0/40(0%)	1/39 (3%)	4/38 11%)
First Incidence	728 (T)	--	--	728 (T)	725
Statistical Tests					
Life Table	p<0.001**	p=0.520N	p=0.510N	p=0.745	p=0.085
Poly 3	p=0.002**	p=0.535N	p=0.524N	p=0.757N	p=0.097
Poly 1.5	p=0.003**	p=0.545N	p=0.535N	p=0.758N	p=0.101
Poly 6	p=0.002**	p=0.525N	p=0.511N	p=0.758N	p=0.093
Logistic Regression	p<0.001**	(e)	(e)	p=0.745	p=0.086
Coch-Arm/Fishers	p=0.003**	p=0.570N	p=0.565N	p=0.752N	p=0.104

Table B-7. Statistical Analysis of Liver Hepatocellular Carcinoma or Hepatocellular Adenoma in Male Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	5/65 (8%)	3/50 (6%)	2/50 (4%)	7/65 (11%)	18/65 (28%)
Poly-3-Rate (b)	5/57.27	3/46.38	2/44.61	7/54.37	18/52.60
Poly-3 Percent (g)	8.7%	6.5%	4.5%	12.9%	34.2%
Int Sacrifice 1 (c)	1/10 (10%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	1/10 (10%)
Terminal (d)	3/43 (7%)	2/35 (6%)	1/39(3%)	5/38 (13%)	14/32 (44%)
First Incidence	549 (I)	723	718	665	549 (I)
Statistical Tests					
Life Table	p<0.001**	p=0.584N	p=0.373N	p=0.310	p<0.001**
Poly 3	p<0.001**	p=0.477N	p=0.328N	p=0.344	p<0.001**
Poly 1.5	p<0.001**	p=0.489N	p=0.336N	p=0.360	p<0.001**
Poly 6	p<0.001**	p=0.465N	p=0.313N	p=0.324	p<0.001**
Logistic Regression	p<0.001**	p=0.495N	p=0.333N	p=0.347	p<0.001**
Coch-Arm/Fishers	p<0.001**	p=0.512N	p=0.341N	p=0.382	p<0.002**

Table B-8. Statistical Analysis of Liver Hepatocellular Carcinoma or Hepatocellular Adenoma in Female Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	1/65 (2%)	1/49 (2%)	0/50 (0%)	2/65 (3%)	8/65 (12%)
Poly-3-Rate (b)	1/53.69	1/45.07	0/47.64	2/54.52	8/53.84
Poly-3 Percent (g)	1.9%	2.2.%	0.0%	3.7%	14.9%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	1/10 (10%)
Terminal (d)	1/42 (2%)	0/38 (0%)	0/40(0%)	2/39 (5%)	4/38 11%)
First Incidence	728 (T)	704	--	728 (T)	550 (I)
Statistical Tests					
Life Table	p<0.001**	p=0.744	p=0.510N	p=0.474	p=0.016*
Poly 3	p<0.001**	p=0.722	p=0.524N	p=0.505	p=0.017*
Poly 1.5	p<0.001**	p=0.711	p=0.535N	p=0.505	p=0.017*
Poly 6	p<0.001**	p=0.736	p=0.511N	p=0.505	p=0.016*
Logistic Regression	p<0.001**	p=0.735	(e)	p=0.474	p=0.018*
Coch-Arm/Fishers	p<0.001**	p=0.677	p=0.565N	p=0.500	p=0.016*

Table B-9. Statistical Analysis of Liver Mass in Male Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	5/65 (8%)	0/50 (0%)	1/55 (2%)	2/65 (3%)	12/65 (18%)
Poly-3-Rate (b)	5/56.91	0/51.36	1/49.61	2/54.13	12/52.30
Poly-3 Percent (g)	8.8%	0.0%	2.0%	3.7%	22.9%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	1/10 (10%)
Terminal (d)	3/43 (7%)	0/40 (0%)	0/44(0%)	1/38 (3%)	10/32 (31%)
First Incidence	658	--	718	713	549 (I)
Statistical Tests					
Life Table	p<0.001**	p=0.042N*	p=0.109N	p=0.270N	p=0.015*
Poly 3	p<0.001**	p=0.041N*	p=0.137N	p=0.238N	p=0.036*
Poly 1.5	p<0.001**	p=0.044N*	p=0.143N	p=0.230N	p=0.045*
Poly 6	p<0.001**	p=0.038N*	p=0.127N	p=0.251N	p=0.025*
Logistic Regression	p<0.001**	p=0.042N*	p=0.134N	p=0.247N	p=0.027*
Coch-Arm/Fishers	p<0.001**	p=0.043N*	p=0.147N	p=0.220N	p=0.058

Table B-10. Statistical Analysis of Liver Mass in Female Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	0/65 (0%)	1/49 (2%)	0/50 (0%)	1/65 (2%)	3/65 (5%)
Poly-3-Rate (b)	0/53.69	1/45.43	0/47.64	1/54.52	3/53.26
Poly-3 Percent (g)	0.0%	2.2.%	0.0%	1.8%	5.6%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	0/10 (0%)
Terminal (d)	0/42 (0%)	0/38 (0%)	0/40(0%)	1/39 (3%)	1/38 (3%)
First Incidence	--	597	--	728 (T)	583
Statistical Tests					
Life Table	p=0.026*	p=0.487	(e)	p=0.485	p=0.116
Poly 3	p=0.035*	p=0.466	(e)	p=0.503	p=0.118
Poly 1.5	p=0.038*	p=0.456	(e)	p=0.503	p=0.120
Poly 6	p=0.032*	p=0.479	(e)	p=0.503	p=0.116
Logistic Regression	p=0.046*	p=0.419	(e)	p=0.485	p=0.122
Coch-Arm/Fishers	p=0.041*	p=0.430	(e)	p=0.500	p=0.122

T**able B-11**. Statistical Analysis of Mononuclear Cell Leukemia (Spleen) in Male Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	**0 ppm**	**500 ppm**	**1500 ppm**	**6000 ppm**	**12,000 ppm**
Overall (a)	22/65 (34%)	23/55 (42%)	21/55 (38%)	32/65 (49%)	30/65 (46%)
Poly-3-Rate (b)	22/57.83	23/52.94	21/51.79	32/58.03	30/58.34
Poly-3 Percent (g)	38.1%	43.5%	40.6%	55.1%	51.4%
Int Sacrifice 1 (c)	1/10 (10%)	0/0 (0%)	0/0 (0%)	0/10 (0%)	0/10 (0%)
Terminal (d)	16/43 (37%)	14/40 (35%)	14/44(32%)	19/38 (50%)	11/32 (34%)
First Incidence	549 (I)	601	505	352	468
Statistical Tests					
Life Table	p=0.002**	p=0.344	p=0.568	p=0.027*	p=0.022*
Poly 3	p=0.047*	p=0.349	p=0.471	p=0.043*	p=0.098
Poly 1.5	p=0.062	p=0.300	p=0.418	p=0.047*	p=0.100
Poly 6	p=0.032*	p=0.415	p=0.556	p=0.041*	p=0.099
Logistic Regression	p=0.054	p=0.337	p=0.407	p=0.035*	p=0.085
Coch-Arm/Fishers	p=0.084	p=0.239	p=0.381	p=0.054	p=0.105

Table B-12. Statistical Analysis of Mononuclear Cell Leukemia (Spleen) in Female Fischer 344 Rats: Terminal Sacrifice at 104 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	6000 ppm	12,000 ppm
Overall (a)	17/65 (26%)	16/49 (33%)	9/50 (18%)	30/65 (46%)	29/65 (45%)
Poly-3-Rate (b)	17/56.56	16/46.45	9/48.14	30/58.71	29/58.34
Poly-3 Percent (g)	30.1%	34.4.%	18.7%	51.1%	49.7%
Int Sacrifice 1 (c)	0/10 (0%)	0/0 (0%)	0/0 (0%)	1/10 (10%)	1/10 (10%)
Terminal (d)	10/42 (24%)	11/38 (29%)	6/40(15%)	16/39 (41%)	15/38 (40%)
First Incidence	433	539	618	443	443
Statistical Tests					
Life Table	p<0.001**	p=0.514	p=0.094N	p=0.020*	p=0.021*
Poly 3	p<0.001**	p=0.396	p=0.130N	p=0.015*	p=0.022*
Poly 1.5	p<0.001**	p=0.348	p=0.157N	p=0.014*	p=0.021*
Poly 6	p<0.001**	p=0.456	p=0.105N	p=0.016*	p=0.024*
Logistic Regression	p=0.002**	p=0.308	p=0.209N	p=0.015*	p=0.022*
Coch-Arm/Fishers	p=0.002**	p=0.291	p=0.209N	p=0.014*	p=0.022*

(a) Number of tumor-bearing animals/number of animals examined at site
(b) Number of tumor-bearing animals/Poly-3 number
(c) Observed incidence at interim sacrifice.
(d) Observed incidence at terminal kill.
(e) Value of statistic cannot be computed.
(f) Beneath the control incidence are the p-values associated with the trend test. Beneath the dosed group incidence are the p-values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. Logistic regression is an alternative method for

analyzing the incidence of non-fatal tumors. The Cochran-Armitage and Fishers Exact tests compare directly the overall incidence rates. For all test a negative trend is indicated by N.
(g) Poly-3 adjusted lifetime tumor incidence.
(I) Interim sacrifice
(T) Terminal sacrifice
Tumor rates based on number of animals necropsied.
* To the right of any statistical result, it indicates significance at $p \leq 0.05$.
** To the right of any statistical result, it indicates significance at $p \leq 0.01$

B. B6C3F1 Mouse

Table B-13. Statistical Analysis of Hepatocellular Adenoma in Male B6C3F1 Mice: Terminal Sacrifice at 105 Weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	10/70 (14%)	7/67(10%)	8/66(12%)	15/65 (23%)	13/70(19%)
Poly-3-Rate (b)	10/57.06	7/56.68	8/53.36	15/52.99	13/50.16
Poly-3 Percent (g)	17.5%	12.4%	15.0%	28.3%	25.9%
Int Sacrifice 1 (c)	1/15(6.7%)	1/14 (7.1%)	3/13 (23.1%)	2/14 (14.3%)	1/15 (6.7%)
Terminal (d)	9/46 (20%)	5/46 (11%)	5/40 (13%)	12/37 (32%)	10/32 (31%)
First Incidence	549 (I)	549 (I)	549 (I)	549 (I)	167
Statistical Tests					
Life Table	p=0.008**	p=0.306N	p=0.527N	p=0.074	p=0.096
Poly 3	p=0.026*	p=0.304N	p=0.459N	p=0.128	p=0.204
Poly 1.5	p=0.039*	p=0.314N	p=0.462N	p=0.127	p=0.244
Poly 6	p=0.016*	p=0.293N	p=0.454N	p=0.131	p=0.163
Logistic Regression	p=0.035*	p=0.313N	p=0.474N	p=0.121	p=0.204
Coch-Arm/Fishers	p=0.079	p=0.338N	p=0.453N	p=0.137	p=0.324

Table B-14. Statistical Analysis of Hepatocellular Adenoma in Female B6C3F1 Mice: Terminal Sacrifice at 105 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	2/70 (3%)	4/68 (6%)	5/68 (7%)	4/67 (6%)	18/70 (26%)
Poly-3-Rate (b)	2/54.79	4/54.03	5/56.52	4/49.67	18/56.96
Poly-3 Percent (g)	3.7%	7.4%	8.9%	8.1%	31.6%
Int Sacrifice 1 (c)	0/15 (0%)	0/15(0%)	1/14 (7.1%)	0/14 (0%)	2/15 (13.3%)
Terminal (d)	2/44 (5%)	4/39 (10%)	2/41(5%)	4/32 (13%)	15/41 (37%)
First Incidence	730 (T)	730 (T)	549 (I)	730 (T)	549(I)
Statistical Tests					
Life Table	p<0.001**	p=0.283	p=0.213	p=0.202	p<0.001**
Poly 3	p<0.001**	p=0.331	p=0.230	p=0.293	p<0.001**
Poly 1.5	p<0.001**	p=0.333	p=0.222	p=0.306	p<0.001**
Poly 6	p<0.001**	p=0.328	p=0.240	p=0.276	p<0.001**
Logistic Regression	p<0.001**	p=0.283	p=0.219	p=0.202	p<0.001**
Coch-Arm/Fishers	p<0.001**	p=0.327	p=0.209	p=0.320	p<0.001**

Table B-15. Statistical Analysis of Hepatocellular Carcinoma in Male B6C3F1 Mice: Terminal Sacrifice at 105 Weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	10/70 (14%)	8/67(12%)	10/66(15%)	17/65 (26%)	20/70(29%)
Poly-3-Rate (b)	10/56.76	8/56.25	10/53.41	17/54.05	20/54.24
Poly-3 Percent (g)	17.6%	14.2%	18.7%	31.5%	36.9%
Int Sacrifice 1 (c)	0/15(0%)	0/14 (0%)	1/13 (7.7%)	2/14 (14.3%)	3/15 (20%)
Terminal (d)	9/46 (20%)	6/46 (13%)	5/40 (13%)	8/37 (22%)	10/32 (31%)
First Incidence	656	633	549 (I)	494	366
Statistical Tests					
Life Table	p<0.001**	p=0.392N	p=0.488	p=0.050	p=0.005**
Poly 3	p<0.001**	p=0.407N	p=0.539	p=0.067	p=0.017*
Poly 1.5	p<0.001**	p=0.416N	p=0.533	p=0.062	p=0.019*
Poly 6	p<0.001**	p=0.395N	p=0.551	p=0.074	p=0.015*
Logistic Regression	p<0.001**	p=0.404N	p=0.511	p=0.057	p=0.019*
Coch-Arm/Fishers	p=0.002**	p=0.440N	p=0.539	p=0.066	p=0.031*

Table B-16. Statistical Analysis of Hepatocellular Carcinoma in Female B6C3F1 Mice: Terminal Sacrifice at 105 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	1/70 (1%)	2/68 (3%)	5/68 (7%)	7/67 (10%)	19/70 (27%)
Poly-3-Rate (b)	1/54.79	2/54.60	5/55.90	7/50.88	19/56.16
Poly-3 Percent (g)	1.8%	3.7%	8.9%	13.8%	32.1%
Int Sacrifice 1 (c)	0/15 (0%)	1/15(6.7%)	0/14 (0%)	0/14 (0%)	2/15 (13.3%)
Terminal (d)	1/44 (2%)	1/39 (3%)	3/41(7%)	1/32 (3%)	8/41 (20%)
First Incidence	730 (T)	549 (I)	640	583	537
Statistical Tests					
Life Table	p<0.001**	p=0.472	p=0.100	p=0.020*	p<0.001**
Poly 3	p<0.001**	p=0.499	p=0.107	p=0.024*	p<0.001**
Poly 1.5	p<0.001**	p=0.498	p=0.104	p=0.026*	p<0.001**
Poly 6	p<0.001**	p=0.499	p=0.111	p=0.022*	p<0.001**
Logistic Regression	p<0.001**	p=0.493	p=0.109	p=0.025*	p<0.001**
Coch-Arm/Fishers	p<0.001**	p=0.489	p=0.098	p=0.027*	p<0.001**

Table B-17. Statistical Analysis of Hepatocellular Carcinoma or Hepatocellular Adenoma in Male B6C3F1 Mice: Terminal Sacrifice at 105 Weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	16/70 (23%)	13/67(19%)	18/66(27%)	28/65 (43%)	31/70(44%)
Poly-3-Rate (b)	16/57.34	13/57.27	18/55.13	28/55.04	31/56.14
Poly-3 Percent (g)	27.9%	22.7%	32.7%	50.9%	55.2%
Int Sacrifice 1 (c)	1/15(6.7%)	1/14 (7.1%)	4/13 (30.8%)	3/14 (21.4%)	4/15 (26.7%)
Terminal (d)	14/46 (30%)	9/46 (20%)	10/40 (25%)	17/37 (46%)	18/32 (56%)
First Incidence	549 (I)	549 (I)	549 (I)	494	167
Statistical Tests					
Life Table	p<0.001**	p=0.336N	p=0.274	p=0.005**	p<0.001**
Poly 3	p<0.001**	p=0.334N	p=0.365	p=0.008**	p=0.002**
Poly 1.5	p<0.001**	p=0.352N	p=0.347	p=0.007**	p=0.002**
Poly 6	p<0.001**	p=0.312N	p=0.395	p=0.010*	p<0.001**
Logistic Regression	p<0.001**	p=0.350N	p=0.313	p=0.007**	p=0.002**
Coch-Arm/Fishers	p<0.001**	p=0.388N	p=0.346	p=0.010*	p=0.006**

Table B-18. Statistical Analysis of Hepatocellular Carcinoma or Hepatocellula Adenoma in Female B6C3F1 Mice: Terminal Sacrifice at 105 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	3/70 (4%)	5/68 (7%)	10/68 (15%)	11/67 (16%)	33/70 (47%)
Poly-3-Rate (b)	3/54.79	5/54.60	10/56.87	11/50.88	33/59.73
Poly-3 Percent (g)	5.5%	9.2%	17.6%	21.6%	55.3%
Int Sacrifice 1 (c)	0/15 (0%)	1/15(6.7%)	1/14 (7.1%)	0/14 (0%)	3/15 (20%)
Terminal (d)	3/44 (7%)	4/39 (10%)	5/41(12%)	5/32 (16%)	21/41 (51%)
First Incidence	730 (T)	549(I)	549 (I)	583	537
Statistical Tests					
Life Table	p<0.001**	p=0.301	p=0.039*	p=0.008**	p<0.001**
Poly 3	p<0.001**	p=0.355	p=0.043*	p=0.014*	p<0.001**
Poly 1.5	p<0.001**	p=0.355	p=0.039*	p=0.015*	p<0.001**
Poly 6	p<0.001**	p=0.354	p=0.047*	p=0.012*	p<0.001**
Logistic Regression	p<0.001**	p=0.347	p=0.041*	p=0.012*	p<0.001**
Coch-Arm/Fishers	p<0.001**	p=0.343	p=0.034*	p=0.018*	p<0.001**

Table B-19. Statistical Analysis of Liver Mass in Male B6C3F1 Mice: Terminal Sacrifice at 105 Weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	14/70 (20%)	18/70(26%)	18/70(26%)	31/70 (44%)	25/70(36%)
Poly-3-Rate (b)	14/57.76	18/60.04	18/57.98	31/60.04	25/55.61
Poly-3 Percent (g)	24.2%	30.0%	31.0%	51.6%	45%
Int Sacrifice 1 (c)	1/15(6.7%)	1/15 (6.7%)	4/15 (26.7%)	4/15 (26.7%)	4/15 (26.7%)
Terminal (d)	11/46 (24%)	14/48 (29%)	10/42 (24%)	19/41 (46%)	13/32 (41%)
First Incidence	549 (I)	430	549 (I)	494	366
Statistical Tests					
Life Table	p<0.001**	p=0.320	p=0.215	p<0.001**	p=0.003**
Poly 3	p=0.002**	p=0.310	p=0.269	p<0.001**	p=0.014*
Poly 1.5	p=0.003**	p=0.300	p=0.264	p<0.001**	p=0.016*
Poly 6	p<0.001**	p=0.320	p=0.280	p=0.002**	p=0.012*
Logistic Regression	p=0.002**	p=0.298	p=0.246	p<0.001**	p=0.018*
Coch-Arm/Fishers	p=0.008**	p=0.273	p=0.273	p=0.002**	p=0.029*

Table B-20. Statistical Analysis of Liver Mass in Female B6C3F1 Mice: Terminal Sacrifice at 105 weeks

Tumor Rates	0 ppm	500 ppm	1500 ppm	4000 ppm	8,000 ppm
Overall (a)	2/70 (3%)	7/70 (10%)	11/70 (16%)	13/70 (19%)	28/70 (40%)
Poly-3-Rate (b)	2/55.36	7/57.17	11/58.41	13/53.65	18/56.96
Poly-3 Percent (g)	3.6%	12.2%	18.8%	24.2%	46.4%
Int Sacrifice 1 (c)	1/15 (6.7%)	2/15(13.3%)	1/15(6.7%)	0/15(0%)	4/15 (26.7%)
Terminal (d)	1/44 (2%)	5/41 (12%)	6/42(14%)	6/34 (18%)	15/41 (37%)
First Incidence	549 (I)	549 (I)	549 (I)	583	537
Statistical Tests					
Life Table	p<0.001**	p=0.073	p=0.011*	p<0.001**	p<0.001**
Poly 3	p<0.001**	p=0.089	p=0.011*	p=0.002**	p<0.001**
Poly 1.5	p<0.001**	p=0.089	p=0.010*	p=0.002**	p<0.001**
Poly 6	p<0.001**	p=0.089	p=0.012*	p<0.001**	p<0.001**
Logistic Regression	p<0.001**	p=0.086	p=0.011*	p=0.002**	p<0.001**
Coch-Arm/Fishers	p<0.001**	p=0.083	p=0.008**	p=0.002**	p<0.001**

(a) Number of tumor-bearing animals/number of animals examined at site
(b) Number of tumor-bearing animals/Poly-3 number
(c) Observed incidence at interim sacrifice.
(d) Observed incidence at terminal kill.
(e) Value of statistic cannot be computed.
(f) Beneath the control incidence are the p-values associated with the trend test. Beneath the dosed group incidence are the p-values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. Logistic regression is an alternative method for analyzing the incidence of non-fatal tumors. The Cochran-Armitage and Fishers Exact tests compare directly the overall incidence rates. For all test a negative trend is indicated by N.

(g) Poly-3 adjusted lifetime tumor incidence.
(I) Interim sacrifice
(T) Terminal sacrifice
Tumor rates based on number of animals necropsied.
* To the right of any statistical result, it indicates significance at $p \leq 0.05$.
** To the right of any statistical result, it indicates significance at $p \leq 0.01$