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Report to the
U.S. Consumer Product Safety Commission
by the

CHRONIC HAZARD ADVISORY PANEL ON DI(2-ETHYLHEXYL)PHTHALATE (DEHP)

SEPTEMBER 1985

U.S. Consumer Product Safety Commission
Directorate for Health Sciences
Washington, D.C. 20207

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REPORT TO THE

U.S. CONSUMER PRODUCT SAFETY COMMISSION

BY THE

CHRONIC HAZARD ADVISORY PANEL

ON

DI(2-ETHYLHEXYL)PHTHALATE (DEHP)

SEPTEMBER 1985

U.S. CONSUMER PRODUCT SAFETY COMMISSION

DIRECTORATE FOR HEALTH SCIENCES

WASHINGTON, D.C. 20207

OAK RIDGE NATIONAL LABORATORY
OPERATED BY MARTIN MARIETTA ENERGY SYSTEMS INC.

POST OFFICE BOX Y
OAK RIDGE, TENNESSEE 37831

September 30, 1985

Terrence Scanlon, Chairman
Consumer Product Safety Commission
Washington, D.C. 20207

Dear Chairman Scanlon:

On behalf of the Chronic Hazard Advisory Panel on di(2-ethylhexyl)-phthalate (DEHP), I am pleased to transmit the Panel's report.

The Panel concluded that DEHP is carcinogenic in mice and rats, causing cancer of the liver. The substance is not mutagenic, however, and belongs to a special class of carcinogens that share the property of inducing proliferation of perioxisomes, an organelle within cells. DEHP is also capable of causing testicular atrophy and teratogenicity in experimental animals. Epidemiologic studies have been inadequate to assess the possible carcinogenic risk to humans from DEHP. As DEHP is an animal carcinogen, it must be considered potentially carcinogenic to humans.

In its report, the Panel has summarized the information available from published reports and from scientists working on this subject. The Panel has also addressed the questions posed by the Commission and provided supporting documentation for the responses. Finally, the Panel has recommended a few, high priority research needs where additional data would permit a fuller, more definitive health risk assessment of DEHP.

The members of the Panel agree with the contents of the report and have indicated their approval below.

Sincerely,

Richard A. Griesemer

Richard A. Griesemer, Chairman
Chronic Hazard Advisory Panel
on DEHP

Approved:

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Curtis Harper, Vice Chairman

Edward Calabrese

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CHRONIC HAZARD ADVISORY PANEL

ON

DI(2-ETHYLHEXYL)PHTHALATE (DEHP)

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Chronic Hazards Advisory Panel (CHAP)
on Di(2-ethylhexyl)phthalate (DEHP)

Report to the U.S. Consumer Product Safety Commission (CPSC)

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I. EXECUTIVE SUMMARY

The DEHP Chronic Hazard Advisory Panel has reviewed the available information on the possible adverse health effects from exposure to the substance di(2-ethylhexyl)phthalate, a chemical added to polyvinyl plastics to make pliable plastic products. The Panel's findings are summarized here and described in more detail in the accompanying text.

- Animal experiments indicate that DEHP is readily absorbed after ingestion and that substantively less is absorbed through the skin. Metabolism is similar in various mammalian species but quantitive differences of unknown importance have been reported. There are no indications that metabolism in humans would differ qualitatively from that in rodents. DEHP and/or its major metabolite, MEHP, reaches all the organs in the body and is rapidly excreted without significant accumulation in the tissues.

- In animals the major targets showing toxic effects of DEHP are the liver, testis, and fetus. DEHP caused enlargement of the liver in rodents characterized by proliferation of a cellular organelle, the peroxisome. Following lifetime oral exposure to DEHP, cancer of the liver developed in experimental animals. In rodents DEHP also caused testicular atrophy and reduced fertility in males, and toxicity and teratogenicity in the fetus when injected into pregnant females.

- DEHP belongs to a special class of carcinogens that share the property of inducing proliferation of peroxisomes and of proliferation of liver cells. A plausible but unproved mechanism of carcinogenesis is

that the peroxisome proliferation causes an imbalance in reactive oxygen species that initiates the carcinogenic process. The excess cancers in the animals were found in the liver, the site of greatest peroxisome response.

- DEHP is not mutagenic in microbial or mammalian test systems. The available data show no evidence for direct damage by DEHP to DNA or to chromosomes.

- Epidemiologic studies have been inadequate to assess the possible human carcinogenicity of DEHP. From definitive animal carcinogenicity studies, however, DEHP must be considered potentially carcinogenic to humans.

- Few data exist on the amounts of DEHP to which people are exposed. All exposure estimates made to date are subject to large variation. Plastics containing DEHP are ubiquitous in the environment and it is likely that everyone has some low level of exposure. In the occupational setting, exposures to DEHP cannot be separated easily from exposures to other substances, so it is unlikely that definitive epidemiological studies will be done in the near future.

- It is possible that exposure to DEHP in the neonatal period might be associated with increased risk because of the opportunity for a longer duration of exposure over a lifetime, the general increased absorbability in the neonatal intestine, the potential for enhanced absorption through the skin, and the increased cell turnover in the

immature neonatal liver. Data are lacking, however, and it is not known whether neonates or infants differ from adults in their potential carcinogenic risk from exposure to comparable amounts of DEHP.

- Quantitative risk estimates of exposure to DHEP, assuming no threshold and based on animal carcinogenicity data and currently available mathematical models, suggest that the contribution of dietary DEHP could represent a substantial portion of total liver cancer deaths in humans (100-150 deaths per year). The risks to dialysis patients and hemophiliacs from intravenous exposures to DEHP are estimated to be 10 to 30 times higher. The added risk due to oral exposure to children's products containing DEHP is estimated as roughly 20-100 deaths per year.

- The Panel recommends that research continue to learn whether peroxisome proliferators, including DEHP, have thresholds for carcinogenicity related to their mechanisms of action. The Panel recommends further that attempts continue to develop more accurate measures of DEHP exposure of neonates and that investigations of the relative susceptibility of children of all ages to the toxic effects of environmental chemicals be intensified. Also needed is the development of mathematical models for materials that may act as late stage carcinogens or tumor promoters.

Below are answers to specific questions which the U.S. Consumer Product Safety Commission has asked the DEHP Chronic Hazard Advisory Panel:

1. Q. Are the results of the NTP bioassay on DEHP adequate and sufficient to conclude that DEHP is carcinogenic to rats and mice?

A. Yes, the Panel concluded that the evidence is sufficient to establish the carcinogenicity of DEHP for rats and mice. The same conclusion was reached independently by an international panel - the Working Group of the International Agency for Research on Cancer (IARC Monographs, Vol. 29, pp. 269-294, 1982).

It should be noted, however, that carcinogens vary considerably in their potency, in the kinds of effects they produce and in the amount and times of exposure required to elicit those effects. The carcinogenic effects of DEHP were restricted to the livers of rats and mice, the two species tested. This implies that a common mechanism may be operating in both species.

2. Q. With regard to the number of animals, the study design, the level of exposure and similar issues, does the NTP bioassay provide an adequate basis for a quantitative risk assessment?

A. The design and conduct of the NTP bioassays were adequate to demonstrate the carcinogenicity of DEHP at the relatively high dietary concentrations tested. Because DEHP is low in acute toxicity, it was possible to administer DEHP to animals at relatively high concentrations (up to 1.2% in the diet).

While the data form a firm and acceptable basis for risk assessment, uncertainty is introduced by the difference between the high dosages at which cancers formed in animals and the lower amounts to which people may be exposed. This uncertainty is common to all estimates of risks to humans when made from animal experimental data.

3. Q. Based on the animal data from the NTP bioassay, and considering the absence of adequate human epidemiological data, should DEHP be considered to pose a risk to humans?

A. The Panel believes that in the absence of adequate data on humans, it is reasonable to regard DEHP as presenting a potential carcinogenic risk to humans.

4. Q. What conclusions, if any, can be reached from the available data about the mechanism by which DEHP causes liver tumors in rats and mice?

A. The available data strongly suggest that the predominant mode of action of DEHP is derived from its properties as a peroxisome proliferator, possibly mediated through imbalanced production of reactive oxygen species. Hepatocellular proliferation induced by peroxisomal proliferators may also contribute to DEHP carcinogenicity. The Panel concludes that these possibilities, although plausible, are not proved.

5. Q. What weight, if any, should be given to the hypothesis that DEHP-induced peroxisome proliferation was responsible for the observed tumors in the NTP bioassay? What relevance does this hypothesis have in evaluating human cancer risks?

A. This hypothesis should be given attention in trying to understand the carcinogenic effect of DEHP in rodent liver. Compounds that induce peroxisome proliferation and hepatic hyperplasia and which have been tested for carcinogenicity have been found to induce liver tumors in the animal bioassays. The induction of peroxisome proliferation has been demonstrated for several species including primates, but the relevance for humans is unproven. The sensitivity of the human liver to DEHP and the similarity of the response between human and rodent tissues need to be further defined.

6. Q. Taking into account all available information about DEHP's mechanism of action, is attainment of a threshold dose level necessary before DEHP will cause liver tumors in rodents?

A. All available models of the mechanism of induction of peroxisome proliferation and hepatic hyperplasia suggest that an effect will occur only after sufficient concentrations of the inducing compound are reached. If DEHP induces carcinogenesis solely as a consequence of induction of peroxisome proliferation and hepatic hyperplasia, then a threshold of DEHP concentration should be necessary for the

formation of liver tumors. If DEHP induces carcinogenesis due to other mechanisms, threshold doses are not likely to exist.

7. Q. Does the available information indicate that DEHP is non-genotoxic and, if so, what are the implications of this finding for carcinogenicity and risk assessment?

A. DEHP is not mutagenic but belongs to a special class of peroxisome proliferators that may be genotoxic through indirect mechanisms. Because DEHP belongs to this special class of materials, questions of genotoxicity may not be germane for risk assessment.

8. Q. What are the implications of the available data concerning differences in DEHP metabolism in rodents at high vs. low doses? What conclusions should be drawn about the comparative metabolism of DEHP in mammals?

A. Differences do exist in the disposition and metabolism of DEHP at high and low doses and therefore, nonlinear effects are expected. Quantitative differences in bioavailability and metabolic profiles have been observed among the different species that have been studied. The implications of these dose and species differences are uncertain because the significance of the different metabolites in the carcinogenic and reproductive effects of DEHP is unknown.

9. Q. Considering the available data on the carcinogenicity, metabolism, and mechanism of action of DEHP, and the data on route and levels of human exposure, can a quantitative assessment of human risk be performed? If so, which risk assessment models are compatible with the available data? Are any models to be preferred as particularly appropriate?

A. The bioassay data indicate that DEHP, at the dosage administered, behaves as a complete carcinogen. The metabolic data cannot at this time be related to the carcinogenic or reproductive effects. There are data which indicate that a possible mode of action of DEHP may involve a threshold phenomenon. Under these circumstances, two approaches to risk assessment can be taken. In view of the carcinogenicity data, a risk assessment model leading to linearity at low doses, such as the linearized, multi-stage model, as recommended by OSTP in its statement on chemical carcinogenesis, is appropriate. If a true threshold phenomenon in DEHP carcinogenesis pertains, then risk assessment would involve defining a NOEL (no observed effect level) or a LOEL (lowest observed effect level) and then applying an acceptable safety factor. The available information does not permit a clear choice between the two approaches, and in the chapter on risk assessment both approaches have been used.

10. Q. Is there evidence that children are likely to be more sensitive in general to chemical carcinogens than adults? If

so, should increased sensitivity of children to DEHP be expected and how should such sensitivity be evaluated qualitatively and quantitatively?

A. Little information exists on the possible greater susceptibility of children to chemical carcinogens. However, studies in neonatal rodents have shown that neonatal liver is more susceptible to genotoxic chemical carcinogens than is adult liver. No data are available on the effect of DEHP in children or on immature experimental animals. It is reasonable to assume that children have a greater response to DEHP, in part because of the potential for longer exposure, the proliferative state of the liver in the young and the potential for high tissue uptake. A risk factor for the effect of age cannot be derived from existing data.

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

A. In the only study reported it was found that 6.9% DEHP applied to the skin was absorbed. No information is available as to how age, skin site of application and/or surface area of application will influence skin penetration. However, because this route of exposure may increase the overall body burden of DEHP and its metabolites, the Panel believes that the

potential from dermal exposure should be considered for future risk assessments.

12-14. Q. Is the available exposure information adequate to permit the panel to estimate, if an estimate is feasible, the probable harm to human health that will result from exposure to the substance?

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

Q. What uncertainties are involved in making such an estimate?

A. Exposure measurements are inadequate to permit accurate estimates of probable harm to humans from exposure to DEHP. If assumptions are made about exposure two methodological approaches may be used:

1. Direct computations of upper bound estimates of carcinogenic risk derived from the multi-stage model (as described in the text);
2. Estimations of "safe" levels from a threshold model.

The major uncertainties in these estimates involve:

a. Exposure levels
b. Extrapolation from animals to humans
c. Choice of dose-response models (and associated safety factors if a threshold model is used)

d. Use of upper bound estimates

e. Dose delivered to the target tissues, through multiple routes of exposure.

II. INTRODUCTION

The Chronic Hazard Advisory Panel (CHAP) on Di(2-Ethylhexyl)Phthalate (DEHP) was convened by the U.S. Consumer Product Safety Commission on January 31, 1985. The mission of the CHAP was to advise the Commission concerning potential chronic hazards associated with DEHP in consumer products. Activities of the CHAP were conducted in accordance with sections 28 and 31 of the Consumer Product Safety Act*, 15 U.S.C. 2077, 2080.

Candidate members for the CHAP were selected by the President of the National Academy of Sciences after a nationwide solicitation. From the twenty-one nominees the U.S. Consumer Product Safety Commission selected seven Panel members who, in turn, chose their own Chairman and Vice Chairman. Under the CPSA Panel members must 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 or receive compensation from or have any substantial financial interest in any manufacturer distributor, or retailer of a consumer product.

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 DEHP) in consumer products. The panel is to determine if

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

DEHP is a carcinogen, mutagen, or teratogen and, if feasible, estimate the probable harm to human health that will result from exposure to DEHP.

The CPSC published a Federal Register Notice on December 22, 1983 which expressed its concern that the current use of DEHP as a plasticizer in children's products may result in a substantial exposure of children to a substance that is reported to cause cancer in animals. The potential health hazard to children is believed to result from exposure to DEHP contained in plastic articles such as pacifiers, squeeze toys, plastic baby pants and the vinyl fabric covering of playpen pads or similar articles. In a prior bioassay conducted by the National Toxicology Program, DEHP was reported to be a carcinogen in two species of animals. The Commission investigated various consumer product uses of DEHP and decided to concentrate its investigations on the potential hazard from children's products. The Federal Register Notice asked this Panel to consider questions such as the eleven questions which were specifically listed. (Federal Register 48 (247): 56629-56630 December 22, 1983). Three additional questions were addressed to the Panel in the CPSC Chairman's letter of January 31, 1985. On February 1, 1985, the CPSC Chairman further clarified the scope of the Panel's activities:

> "While children's products are the Commission's primary concern at this juncture, the Commission convened this CHAP to focus on the chemical <u>substance</u> DEHP in consumer products. Nothing, of course, would preclude the CHAP from considering any and all studies or data available with regard to DEHP in reporting to the Commission as to the risk presented by the <u>substance</u> DEHP".

During the eight months following the convening of the Panel, it met four times in open sessions (January 31-February 1, April 3-4, May 9-10, and June 10-11, 1985). Scientific experts were invited to two meetings to address specific areas of interest. A public meeting was held on April 3, 1985 to hear and receive written comments. The Panel advertised and distributed a draft of its report for scientific peer review and public comment prior to final editing and issuance of its report to the Commission.

Since DEHP is a substance of interest to several Federal agencies, members of the Environmental Protection Agency, the Food and Drug Administration and the Occupational Safety and Health Administration were invited to and often did attend the meetings. In order to increase the utility of the report, the staff of the Safe Drinking Water Committee, National Research Council, National Academy of Sciences, which is preparing a report that will include DEHP, was also invited to attend the meetings.

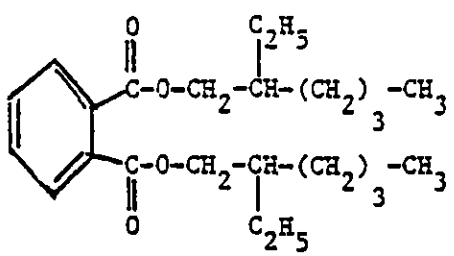
This final report reflects the Panel's response to comments received from the public, industry, and other Federal agencies which share an interest in the state-of-the-science for the health effects of DEHP.

III. ABSORPTION, DISTRIBUTION, AND METABOLISM

A wide spectrum of application has been found for phthalate esters. However, the largest use of phthalate esters is as plasticizers, particularly for polyvinylchloride products. Although all phthalate esters have been used as plasticizers in different applications, di(2-ethylhexyl)phthalate (DEHP) is the most widely used. Recent estimates indicate that the annual production of phthalate esters in the United States is approximately 1-2 x 10^9 lb (Bell 1982).

A. DEHP Chemistry

Di(2-ethylhexyl)phthalate (DEHP) is considered to be synonymous with bis(2-ethylhexyl)phthalate and has also been referred to as dioctyl phthalate (DOP). It has the following structure:



DEHP

It is produced commercially by the reaction of excess 2-ethylhexanol with phthalic anhydride in the presence of a catalyst. DEHP is a long and branched chain ester and it resides as a high mol. wt. homologue in the pthalic acid ester (PAE) series. The boiling point of DEHP is about 387°C and relative to other PAEs, it has a low vapor pressure. Its molecular weight is 391. Industrial processes involving the synthesis

of PAEs yield a purity of over 99%. The main impurities are isophthalic and terephthalic acids and maleic anhydride (Thomas et al 1978). While these impurities are present in only trace amounts, their contribution to toxic manifestations cannot be completely dismissed. Maleic anhydride was not detected (limit of detection 0.005%) in the DEHP used in the NTP bioassays (p. 96).

DEHP is extremely insoluble in water but is soluble in organic solvents and oils. It has an octanol/water partition coefficient of 9.64 (Leyder 1983). It is also soluble in blood and other lipoprotein-containing materials.

Plasticized polyvinyl chloride (PVC) is one of the more widely used plastics. Its flexibility, clarity and toughness, in addition to its low cost, are some of the reasons for its use in a variety of materials, including childrens' products such as teethers and pacifiers. PVC used for these purposes usually contains a very high percentage (as much as 40%) of plasticizer; DEHP is the one most commonly used.

One of the primary factors to be assessed is the leachability of DEHP from its PVC formulations. Since DEHP has good solubility in lipid-like materials, and is not bound in the polymer, the presence of fats, oils, lipoproteins, alcohol or organic solvents, and probably milk, will markedly increase DEHP leachability.

It should also be pointed out that in commercial products, a stabilizer (usually an organometallic compound) and other additives, such as antioxidants, colorants, etc., are present. When studying the adverse biologic properties of DEHP, the presence of these substances should be considered (Lawrence 1978).

B. Absorption Through the Oral Cavity

There are no data available for the absorption of DEHP from the oral cavity. The oral cavity is lined with stratified squamous epithelium like the surface of the skin, not with the simple columnar epithelium of the type that lines the gastrointestinal tract. Saliva, which is directly in contact with the surface of the oral cavity, maintains it in a moist state. The saliva and the stratified squamous epithelium perform an important barrier function against absorption from the oral cavity. However, the hydration of the epithelial lining tissue may increase the permeability of the oral mucosa (Squier and Johnson 1975). There are different mechanisms by which xenobiotics penetrate the epithelial tissue. These include endocytosis, active transport, intercellular movement and diffusion. Several factors affect these processes of penetration of substances through oral mucosa. Most important are the physicochemical properties of the compound. Among these, experimental evidence indicates that small molecules are more penetrating than large molecules, un-ionized molecules are more penetrating than their ionized forms, and volatile substances and gases are highly penetrating through the oral mucosa. In addition, the oil/water partition coefficient of the compound plays a significant role in the oral mucosa absorption processes. Compounds with high lipid solubility and at least some limited aqueous solubility penetrate more readily because of the lipid-aqueous nature of the cell membrane. For ions, the pK value and their relative ionization in the oral cavity is the most significant factor that governs their penetration. Although, as mentioned before, small molecules generally pass through the oral

mucosa more readily than large molecules, this may depend on the experimental design. Dextrose (mol. wt. 70,000) crossed the non-keratinized rabbit mucosa in vitro while in vivo the protein horse radish peroxidase (mol. wt. 40,000) failed to pass (Squier and Johnson 1975). In addition, the solvent in which the compound is dissolved plays a role in oral absorption. Solvents that tend to increase the hydration state of the oral mucosa promoted higher penetration of the compounds applied and the presence of saliva accounts for the increased penetration of xenobiotics to oral mucosa relative to dry skin. In - humans, comparison of the penetration of compounds through oral mucosal surface, skin and gastrointestinal tract after oral administration indicated that, for most compounds, gastrointestinal absorption is higher than absorption through the oral mucosa or outer skin surface. This is to be expected because of the large surface area, with specialized microvilli, that is present in the intestinal tract. At the same time, it is evident that the absorption through hydrated surface skin is similar to the absorption through oral mucosa (Squier and Johnson 1975).

Siegel et al (1976) compared the extent of oral mucosal transfer of alcohols and ureas by using the lingual fenulum of adult male mongrel dogs, in vitro. The rate of transfer of the smallest members of both series (methanol and urea) was greater than ethanol and methyl urea respectively, while the oil/water partition coefficient was higher in case of ethanol and methyl urea. On the other hand, in the alcohol series, as the alcohol chain increased after C_2, the oil/water partition coefficient increased along with the rates of mucosal transfer, while the rates of mucosal transfer of ethyl-and propyl-urea were less than

that of methyl urea (Siegel et al 1976). Therefore, the oral cavity should be considered as a potential site for absorption of DEHP and its hydrolysis products, MEHP and ethylhexanol.

C. Dermal Absorption

Little information is available on the dermal absorption of phthalate esters. However, in a recently reported study by El Sisi et al (1985) a number of phthalates including DEHP were applied to the backs of F-344 male rats (200±20 gm). The site of application was a shaved area of 1.8 cm in diameter. Following application of a single dose (30 mg/Kg) of ^{14}C-phthalate (dissolved in 0.2 ml of ethanol) uniformly labeled on the ring, the area was covered with a perforated cap that was secured by cyanoacrylate glue. The degree of absorption of DEHP was estimated from the cumulative daily excretion of ^{14}C-DEHP equivalents in the urine and feces over five days. At termination, radiolabel was determined in a number of tissues, the site of application and the plastic cap. The results for DEHP are presented in Tables III-1 and III-2. Only 5.1% of the dose was excreted over five days with 3.0% appearing in the urine and 2.1% in the feces. An additional 1.8% of the dose was found in the tissues, with muscle containing 1.2% of the dose. Tissues which contained the highest concentration of ^{14}C-DEHP equivalents (ug/gm) were liver, muscle and skin (at about 0.6 ug/gm). Of the tissues studied, the testis had the lowest concentration at 0.05 ug/gm. Total recovery in the excreta and in the tissues was 6.9% of the dose. The vast majority of the dose remained at the site of application - 87%. Therefore, it was concluded

Table III-1

Excretion Profile of DEHP in the Urine and the

Feces After Dermal Application to the Rat*

	% Dose Excreted		Sum
Time (hr)	Urine	Feces	Urine & Feces
24	0.28 ± 0.07	0.14 ± 0.02	0.42
48	0.57 ± 0.10	0.20 ± 0.16	0.77
72	0.64 ± 0.22	0.29 ± 0.26	0.93
96	1.00 ± 0.60	0.69 ± 0.33	1.69
120	0.50 ± 0.07	0.80 ± 0.35	1.30
Total	2.99 ± 1.05	2.12 ± 0.74	5.11

*Male F-344 rats (200 ± 20 gm) received DEHP in ethanol topically to the skin (30 mg/Kg). The skin was covered with a perforated plastic cap. Data points are the mean ± standard deviation. The % dose excreted represents the faction of the dose found as the ^{14}C-equivalents relative to the total ^{14}C-equivalents applied.

Table III-2

Tissue Distribution and Excretion of ^{14}C-DEHP Radioactivity in the Rat After Single Dermal Exposure to 30 mg/Kg for 5 Days, as Measured on the 5th Day

Tissue	% dose found	ug/gm tissue
Brain	0.006 ± 0.005	0.21
Lung	0.005 ± 0.006	0.27
Liver	0.063 ± 0.017	0.61
Spleen	0.001 ± 0.0003	0.16
Small Intestine & Intestinal Content	0.161 ± 0.158	0.75
Kidney	0.012 ± 0.001	0.42
Testis	0.002 ± 0.001	0.05
Fat	0.066 ± 0.028	0.28
Muscle	0.162 ± 0.22	0.69
Skin	0.298 ± 0.286	0.55
Spinal Cord	0.002 ± 0.0001	0.32
Blood	0.023 ± 0.001	0.07
Skin area of application	86.76 ± 16.98*	-
Urine	2.99 ± 1.05	-
Feces	2.12 ± 0.76	-

* % of administered material still present on the skin and available for absorption after 5 days.

A single application of 30 mg/Kg of ^{14}C-DEHP was applied to a clipped area of skin and then covered with a perforated cap. After five days the animals were sacrificed and the % dose ± S.D. (as ^{14}C-equivalents) excreted and that present in various tissues and at the site of application was determined.

that DEHP was poorly absorbed when applied to the skin of rats. That fraction of dose which was absorbed was readily eliminated. The fact that 87% of the dose remained at the site of application insured the availability of the compound for continuous exposure over the five day period.

The nature of the DEHP that is absorbed from the skin is unknown. Mammalian skin posseses cytochrome P-450 activity and has been shown to metabolize benzo(a)pyrene and related compounds. In addition, skin is known to possess epoxide hydrolase and glutathione-s-transferase activity (Bickers et al 1981). The ability of mammalian skin to de-esterify xenobiotics in vivo is not clearly established and the existence of non-specific lipases and esterases in the skin has not been verified, to our knowledge. Albro et al (1984) did show that a homogenate of rat skin possessed DEHP hydrolase activity. This activity was equivalent to hepatic activity, when expressed as U/mg protein. However, when expressed as U/g tissue, skin possessed only 1% of the activity reported for liver.

A number of factors are known to affect the percutaneous absorption of xenobiotics. These include dose, the site of application, its surface area, skin condition and occlusion among others (Wester and Maibach 1983). The data in Table III-3 illustrate how the site of application can affect the penetration ratio of hydrocortisone applied topically. Note the high penetration for the scrotal area. There is a direct relationship between the dose and surface area of application, and the extent of percutaneous absorption. Damage to the skin, in which normal protective barriers are compromised, usually results in enhanced

Table III-3

Penetration of Hydrocortisone in Human Skin
After Application to Various Anatomic Sites

Anatomic Site	Penetration Ratio*
Forearm (ventral)	1.0
Forearm (dorsal)	1.1
Foot	0.14
Ankle	0.42
Palm	0.83
Back	1.7
Scalp	3.5
Axilla	3.6
Forehead	6.0
Jaw angle	13.0
Scrotum	42.0

From Wester and Maibach 1983 (after Feldman and Maibach 1967).

*Penetration Ratio =

$$\frac{\text{Total urinary excretion after application to each anatomic site}}{\text{Total urinany excretion after application to the ventral forearm}}$$

percutaneous absorption. Furthermore, occlusion of the exposed area increases absorption from the skin by increasing the hydration state of the skin and skin temperature and by preventing loss by physical means and evaporation. Of course, the properties of the compound can be important determinants of absorption. This was apparent in studies by El Sisi et al (1985) that determined the effect of increasing chain length on the dermal absorption of phthalate esters. The symmetrical

diesters included: dimethyl-, diethyl, dibutyl-, diisobutyl-, dihexyl-, di(2-ethylhexyl)-, and diisodecyl-phthalate. Absorption increased as the chain length increased up to C_4(dibutyl phthalate). As chain length was further increased, dermal absorption decreased. The total recovery of ^{14}C-equivalents of dimethyl-and diethyl-phthalates was lower than that of the higher molecular weight homologues, suggesting loss of these more volatile agents by evaporation (even in the presence of an occluding perforated cap). Decreased absorption of the higher homologues probably resulted from dramatically reduced solubility and steric factors.

D. Absorption and Distribution of DEHP Following Oral Administration

The fate of orally administered DEHP has been studied in more detail than that of topically applied DEHP. After oral administration, DEHP undergoes hydrolytic degradation in the small intestine to mono(2-ethylhexyl)phthalate (MEHP) and 2-ethylhexanol (EH) in all animal species studied (Albro 1984, Daniel and Bratt 1974, Albro 1983). This metabolic step has been shown to be mediated by the pancreatic non-specific lipase (DEHP hydrolase activity) (Albro 1973), which has high activity and can result in total degradation of DEHP to MEHP and EH in the intestine. Among the rodents, pancreatic DEHP hydrolase activity is affected to some extent by sex and species. Age does not seem to be a factor, since Albro et al (1973) found no significant difference in DEHP-hydrolase activity between young adult and old rats. In this study by Albro (1973) mice showed the highest activity in units/mg protein (4.01 ± 0.6) followed by rats (1.7 ± 0.8), guinea pigs (1.2 ± 0.4) and

hamsters (0.36 ± 0.2). Male rats showed higher DEHP hydrolase activity than female (0.45 ± 0.03). Both MEHP and EH were absorbed via the intestine without further alterations and it was shown that MEHP is not a substrate for pancreatic non-specific lipase (Albro 1973). Following oral administration of EH to rats in doses of 30 ug/Kg to 300 mg/Kg absorption was complete. After 28 hours, the excretion of EH equivalents was much higher in the urine (80%) than in the feces (8.6%) and in exhaled CO_2(5%). The carcasses retained about 1.1% of the administered dose (Albro 1975). The urinary excretion of EH in this study was interesting because a burst of ^{14}C excretion, which represented 41% of the dose, appeared in the urine between 11 and 12 hours following administration (Albro 1975). It has been shown that the only enzyme capable of de-esterification of MEHP to phthalic acid at a significant rate is the alkaline liver lipase, an enzyme that shows low DEHP hydrolase activity compared to the other liver DEHP hydrolase activities (Albro 1973). However, it metabolizes MEHP to phthalic acid at 2% of its activity to metabolize DEHP to MEHP. This observation is in agreement with the finding that only 2-3% of the urinary metabolites of DEHP is excreted as free phthalic acid.

There is a remarkable dose effect on the extent of intact DEHP absorption after single oral administration in the rat and the mouse. In the Fisher rat a relatively discrete threshold for oral absorption of intact DEHP was observed. Below a dose of 0.42 g/Kg intact diester did not reach the liver, but above this dose there was a rapidly increasing dose dependent absorption of intact diester (Albro 1982). This threshold effect also has been observed in CD-1 mice but with a slightly higher threshold dose (0.5 g/Kg, Albro 1985b). This high dose

threshold may be attributed to a higher activity of pancreatic non-specific lipase in mice (Albro 1973). However, this threshold does not seem to exist in B6C3F1 mice. This strain showed a nearly linear relationship between the administered dose of DEHP and the appearance of DEHP in the liver (Albro 1985b). No threshold effect was observed when female Wistar rats were fed diets containing 1000 ppm or 5000 ppm DEHP. Intact DEHP was found in the liver, fat, heart and brain within the first week and reached an apparent steady state for the following four weeks. The concentration of DEHP present in these tissues increased as the dietary concentration increased (Daniel and Bratt 1974). Fecal excretion of unabsorbed DEHP is dose related. Albro (1982) reported that in the rat, DEHP starts to appear in the feces due to non-absorption when the oral dose exceeds 200 mg/Kg.

Oral administration of a single dose of DEHP (100 mg/Kg) to the male rat (F-344), monkey (cynomolgus) and mouse (B6C3F1) resulted in rapid elimination of DEHP equivalents in the first 24 hours (73%, 48%, 82% of the dose respectively). In the rat and mouse, the % dose excreted in the feces after 24 hours of oral administration of DEHP (43.4%, 48.4% respectively) was higher than the % dose excreted in the urine (28.7%, 33.9%). On the other hand, the 24 hour fecal and urinary elimination of DEHP equivalents in the monkey were approximately similar (25%, 23% respectively). However, after 72 hours the cumulative fecal elimination of DEHP equivalents was higher than urinary elimination in all the species studied (Arthur D. Little, 1983). This is in contrast to results obtained for MEHP. When given by oral gavage in a dose of 69

mg/Kg to male SD rats, 72% of the dose of MEHP was excreted in the urine and 8% in the feces during the first 24 hours (Chu et al 1978).

The tissue distribution of DEHP equivalents after oral dosing seems to be somewhat species dependent. In monkeys at 96 hours after a single oral dose (100 mg/Kg), the highest concentration of DEHP equivalents was in the liver (26.9 ± 45.ug/g). At these same doses and times in the rat, intestinal contents retained the highest concentration (5.0 ± 3.2 ug/g), while in the mouse the adipose tissue retained the highest concentration (0.7 ± 0.4 ug/g)(Arthur D. Little 1983). In all other - tissues in the monkey and the rat, the concentration of DEHP equivalents did not exceed 1 ug/g. In 96 hours the % dose excreted in the urine of the monkey, rat and mouse were 28.2 ± 14.7, 32.9 ± 4.8 and 37.2 ± 9.2 respectively. In addition, the % dose excreted in the feces were 49.0 ± 4.6, 51.4 ± 7.8 and 52.0 ± 7.7 respectively. The tissues retained only 0.6 ± 0.8, 0.7 ± 0.5 and 0.1 ± 0.1 % of the dose in the monkey, rat and mouse respectively.

At 24 hours after a single oral administration of DEHP to male cynomolgus monkeys, F-344 rats and B6C3F1 mice, there were quantitative and qualitative differences in the amount of DEHP equivalents excreted in the urine (Arthur D. Little 1983). In the monkey, metabolites V, IX, and MEHP represented 5.7%, 4.1% & 2.5% of the administered dose respectively. In the rat, metabolites V, IX and MEHP represented 8.4%, 5.2% and 0% of the administered dose respectively. In the mouse, metabolites V, IX and MEHP represented 0.4%, 3.7% and 5.8% of the administered dose respectively. At the same time, the mouse excreted 4.4% of the dose as phthalic acid, while the monkey and rat excreted 0.5% and 0.6% of the dose as the acid, respectively. (Metabolites are

numbered according to the convention of Albro 1982). In this study (Arthur D. Little, 1983) the fecal elimination of DEHP equivalents also showed quantitative and qualitative differences according to the animal species. Intact DEHP was found in the feces of the monkey, rat and mouse as 34.3%, 20.0% and 16.5% of the administered dose (100 mg/Kg) respectively. At the same time, MEHP was found in the feces of the mouse as 24.2% of the dose relative to 8.2% in the rat and 3.2% in the monkey. The effect of the dose and prior exposure to DEHP on its excretion has been studied (Arthur D. Little 1982). Without prior exposure to DEHP, administration of 1000 ppm, 6000 ppm or 12000 ppm in the diet to the rat resulted in a 24 hour urinary excretion of 36.2, 45.4 & 49.9% of the dose, respectively. However, the % dose excreted on the 4 days was 52.9, 62.0, and 65.5, respectively. Prior exposure to DEHP at the same dose level/day for 6 or 20 days did not result in a significant change in the % dose excreted in the urine. On the other hand, prior exposure to DEHP for 6 or 20 days resulted in a decreased % dose excreted in the feces. These data suggest increased absorption and tissue retention.

When DEHP was given orally to the rat and marmoset at a dose of 1.96 g/Kg/day for 14 days, species and sex differences in tissue retention were clear (Bratt and Batten 1982). In the rat, the female retained higher concentrations of DEHP equivalents in the liver and kidney (286 ug/g, 176 ug/g tissue respectively) than the male (21 ug/g, 115 ug/g tissue respectively). In addition, male rats retained 36 ug/g tissue in the testis. Furthermore, the female marmosets retained higher concentration of DEHP equivalents in the liver and kidney (47 ug/g and 35 ug/g respectively) than the male marmosets (29 ug/g, and 15

ug/g respectively) which also retained 8 ug/g in the testis. It is also clear that rats retained higher tissue concentrations of DEHP equivalents than marmosets (Bratt and Batten 1982). The effect of the administered dose on the amount of DEHP equivalents retained in the tissue has been further investigated by Arthur D. Little, (1982) in the rat. In this study, the data suggested that there is a linear relationship between the administered dose and the amount of DEHP equivalents accumulated in the tissue. In all the species studies, the nature of the retained DEHP equivalents in the tissues was not determined. Rhodes et al (1985) compared the blood and tissue levels of DEHP equivalents in the rat and marmoset, after multiple oral dosing of 2000 mg DEHP/Kg/day for 14 days. This study indicated that the level of DEHP equivalents in the marmoset tissues was 1/5 - 1/10 of those in the rat tissue. In both rat and marmoset, the liver retained the highest concentration of DEHP equivalents. At the same time, the rat excreted 50% of the dose in the urine compared to 2% in the marmoset in 24 hours. The remainder of the dose was found mainly in the feces. This enhanced fecal elimation of DEHP equivalents in the marmoset probably reflects unabsorbed chemical species and has been interpreted as lower bioavailability of DEHP in the marmoset than in the rat after oral administration (Rhodes 1985).

The ability of DEHP equivalents to pass the placenta has been reported by Singh et al (1975). In this study, when DEHP was administered to pregnant female SD rats (4.97 g/Kg, i.p.) on the 5th and 8th days of gestation, DEHP equivalents appeared in the fetal tissue within 24 hours. The fetal tissue and amniotic fluid contained 0.033 and 0.016% of the injected dose, respectively, while maternal blood

contained 0.29% of the dose. The % dose was followed in the fetal tissue and amniotic fluid for 10 days and compared to the maternal blood contents of DEHP equivalents, which were always higher in the maternal blood (Singh et al 1975). In another study the guinea pig placenta was perfused with heparinized blood containing DEHP. A linear relationship between perfusion time and the amount of DEHP that appeared in the fetal circulation was found (Kihlstrom 1983). Thus, the DEHP equivalents that pass the placenta may be responsible for the fetotoxicity associated with DEHP administration (Kihlstrom 1983).

E. Metabolism of DEHP

1. General Metabolism

The metabolism of DEHP involves a complex network of pathways and secondary metabolites. It is to the credit of most people working in the field that they have adopted the nomenclature of Albro et al (1981).

The urinary metabolites of DEHP have been separated and identified by Albro et al (1983) using thin layer and gas liquid chromatography. The first step in the metabolism is the lipolytic cleavage of DEHP to form two products, 2-ethylhexanol and mono(2-ethylhexyl)phthalate (MEHP). Both of the products undergo extensive metabolism (Albro et al 1983).

The lipolytic enzymes that cleave DEHP have been studied by Albro and Thomas (1973). The DEHP hydrolase enzymes are believed to be the same or very similar to the traditional lipases (lipoprotein lipase).

The DEHP lipase activities were observed in rat liver, lung, kidney, mucosal, pancreas, and adipose tissue. The rapid rates of metabolism by the intestinal mucosa suggested that orally ingested DEHP would have little opportunity to be absorbed intact. The pancreas is the richest tissue by far in enzymes responsible for the hydrolysis of DEHP to MEHP and 2-ethylhexanol.

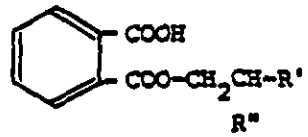
Only one of the tissues was able to further hydrolyze MEHP to phthalic acid. The alkaline lipase of rat liver hydrolyzed MEHP at 2% of the rate at which it hydrolyzed DEHP. This is consistent with the finding that free phthalic acid amounted to less than 3% of the total urinary metabolites. The major metabolites were all derivates of MEHP and 2-ethylhexanol.

The metabolism of 2-ethylhexanol in rats was studied extensively by Albro (1975). The presence of substrate-derived CO_2 in the expired air of the rats, together with the identification 2-and 4-heptanones in the urine suggested that some of the 2-ethylhexanol was metabolized via the oxidative carboxylation of 2- and 4-oxy-3-carboxylheptane. The primary excreted metabolite was 2-ethylhexanoic acid. Several keto acids were found in the urine and were presumed to be beta-oxidation products of the primary metabolite.

The second product of the lipolytic hydrolysis of DEHP is MEHP. Oxidative metabolism of MEHP occurs via w and w-1 oxidation of the aliphatic side chain in a manner analogous to the mixed function oxidase-dependent oxidation of fatty acids (Albro et al 1983). It is believed that this step is followed by a dehydrogenase-dependent oxidation to the ketone or carboxylic acid, with subsequent alpha and beta-oxidation of the acids. In addition to these pathways, it is

Table III-4

Structure of DEHP Metabolites



METABOLITE #	R'	R"
I	$-CH_2COOH$	CH_2CH_3
II	$-CH_2CH_2CH_2CH_3$	COOH
III	$-CH_2CH_2COOH$	CH_2CH_2
IV	$-CH_2CH_2CH_2CH_3$	CH_2COOH
V	$-CH_2CH_2CH_2COOH$	CH_2CH_3
VI	$-CH_2CH_2COCH_3$	CH_2CH_3
VII	$-CH_2CH_2CH_2CH_3$	CH_2CH_2OH
VIII	$-CH_2CHOHCH_2CH_3$	CH_2CH_3
IX	$-CH_2CH_2CHOHCH_3$	CH_2CH_3
X	$-CH_2CH_2CH_2CH_2OH$	CH_2CH_3
XI*	$-CH_2CH_2CH_2CH_3$	CH_2CH_3
XII	$-CH_2COCH_2CH_3$	CH_2CH_3
XIV*	$-CH_2COCH_2CH_3$	CH_2COOH
XV	$-CH_2COCH_2COOH$	CH_2CH_3
XVI	$-CH_2CHOHCH_2COOH$	CH_2CH_3
XVII	$-CH_2CH_2CH_2CH_3$	$CHOHCH_3$
XVIII	$-CH_2CHOHCH_2CH_3$	CH_2COOH
XIX	$-CH_2CH_2CHOHCH_3$	$CHOHCH_3$
XX	$-CH_2CHOHCH_2CH_2OH$	CH_2CH_3
XXI	$-CH_2CH_2CHOHCH_3$	CH_2COOH
XXV	$-CH_2CH_2COCH_3$	CH_2COOH
XXVI	$-CH_2CH_2CH_2CH_3$	$COCH_3$

* Metabolite XI is MEHP; Metabolite XIII, phthalic acid, is not depicted.

Source: Albro et al (1983)

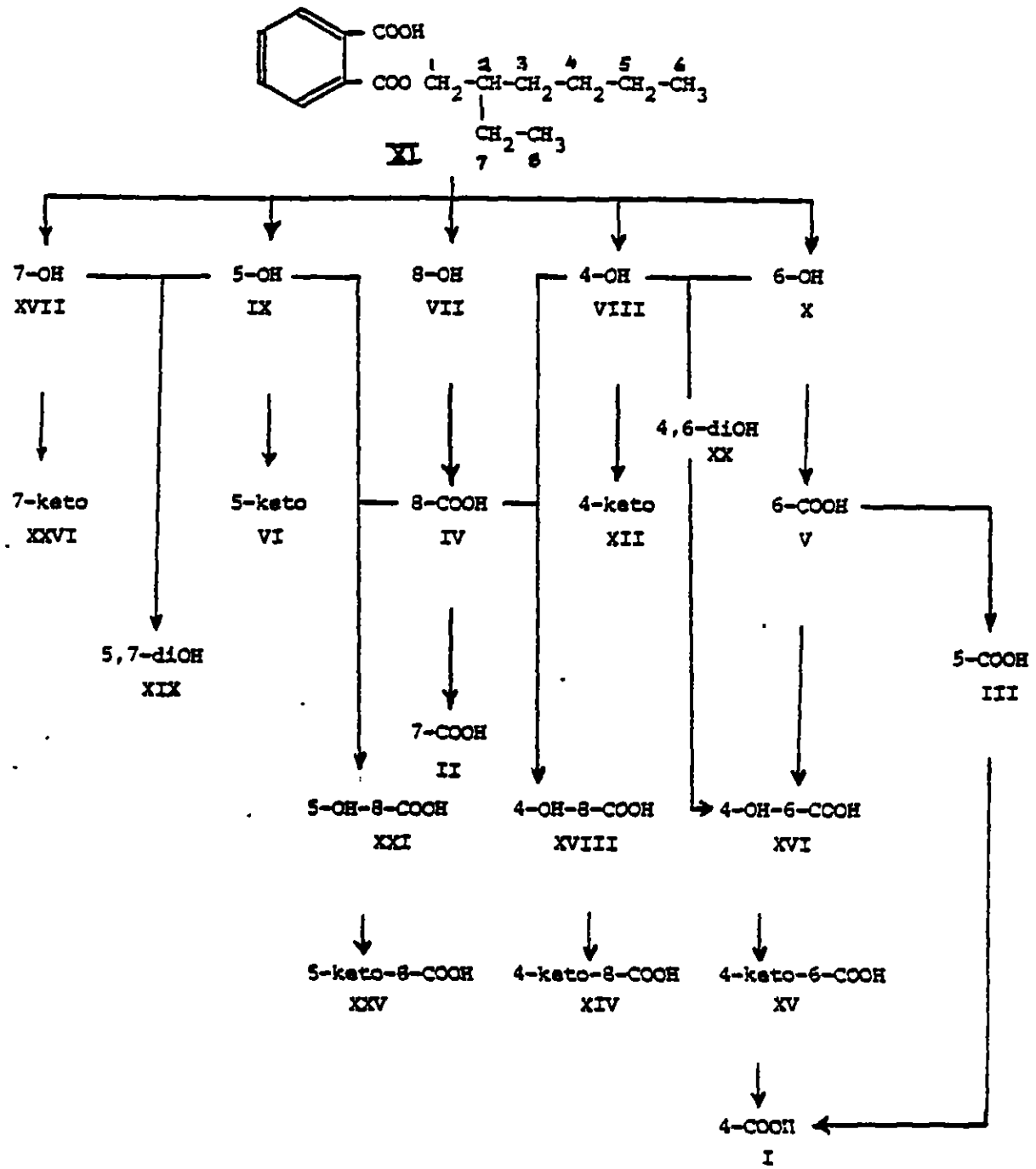


Figure III-1. Hypothetical Pathways in the Formation of MFHP Metabolites

Source: Albro at al (1983).

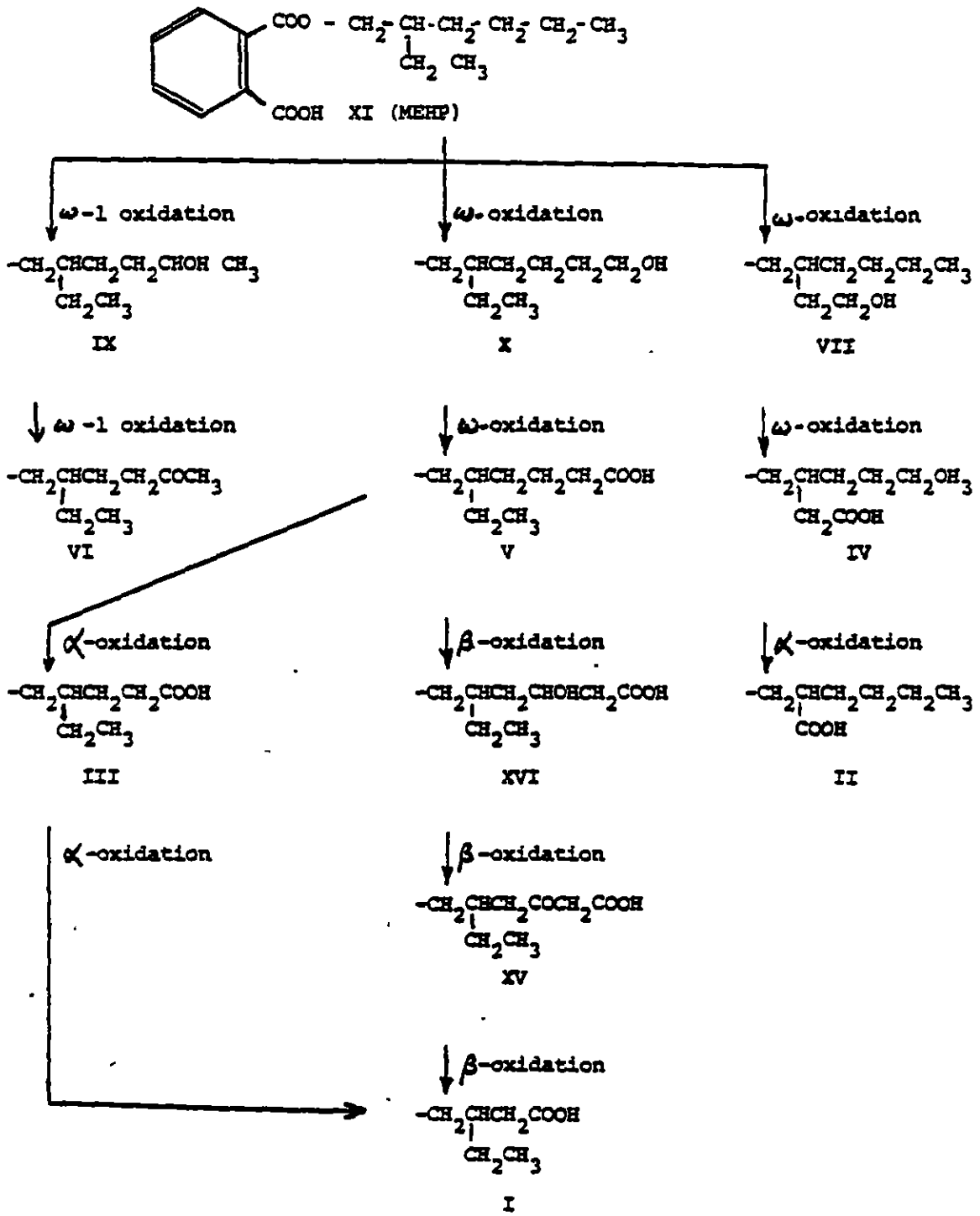


Figure III-2. Hypothetical Pathways in the Formation of DEHP Metabolites

Table III-5

Urinary Metabolites of DEHP Expressed as Percentages of Total Urinary Radioactivity Excreted in the First 24 Hours after Dosing [1]

Metabolite[2]	Monkey	Rat	Mouse[3]
MEHP	11	-[4]	17
Phthalic Acid	2	2	13
I	0.5	11	13
II	-	0.9	0.8
III	0.5	-	0.8
IV	0.5	4	3
V	25	29	1
VI	1	11	12
VII	7[5]	3	6
IX	18	18	11
X	9	4	2
XII	2	6	5
XIII	6	6	7
Uncertain	15	1	5
XIV	2	1	2
Uncertain	1	1	1

[1] From A.D. Little (1983).

[2] Metabolites are numbered according to the convention of P.W. Albro.

[3] The mouse urine extract analyzed by HPLC contained only 79% of the radioactivity excreted in 0-24 hr. The remainder of the radioactivity was eluted from the SAC-2 resin in the acidic aqueous wash, and probably contained some of the more polar metabolites, perhaps including glucuronides.

[4] Radioactivity in the sample was less than twice background for the system.

[5] This fraction may include metabolite VIII, which was identified in monkey urine after i.v. administration of DEHP (Albro et al 1981).

likely that hydroxylation can occur at positions more distant than w-1 from the methyl terminus. The metabolite profile is shown in Table III-4 and the hypothetical metabolite pathways leading to these products is shown in Figures III-1 and III-2.

2. Species Difference in Metabolism

Systematic studies on the species differences in metabolism of DEHP have not been published. An unpublished study was made available to the CHAP by the Chemical Manufacturers Association (Arthur D. Little, 1983). Cynomolgus monkeys, male Fischer-344 rats and male B6C3F1 mice were compared with respect to metabolism of DEHP. The findings are summarized in Table III-5. A primary feature of these findings is that MEHP is not excreted as a metabolite in 1-24 hour urine samples of the rat. This is consistent with the earlier finding by Albro et al, that MEHP is rapidly metabolized in the rat. In contrast, MEHP is 11 and 17% respectively, of the 1-24 hour urinary products in monkeys and mice exposed to DEHP. On the other hand, metabolite I is negligible in the monkey but very prominent in the rat and mouse. The metabolite profiles in the three species do not follow a pattern that marks a clear distinction between rodents and primates. It is useful data that will be better understood when target organ toxicities can be related to metabolism by identifying the toxic intermediate produced at those sites.

3. Human Metabolism

Draviam et al 1982, analyzed urine for DEHP and MEHP from non-uremic psoriatic patients, uremic patients undergoing hemodialysis

treatment, and patients undergoing cardiac bypass surgery. The levels of phthalic acid and DEHP found in the urine of patients on total body oxygenators (which were fitted with membrane containing DEHP) during cardiac bypass surgery were similar to levels found in non-uremic psoriatic patients. Significant levels of phthalic acid were detected in the urine of the uremic patients undergoing dialysis, while DEHP and MEHP were present only in small amounts or were completely absent. It is difficult to draw conclusions about the human metabolism of DEHP based on this single study with a small number of patients. This is especially true since no effort was made to establish a complete metabolite profile as had been done with animal species.

4. Biological Effects of Metabolites

There is little doubt that the adverse biological effects of DEHP are mediated by metabolites rather than by the parent compound. The pathways leading to the metabolites of DEHP are merely postulates based on identification of urinary products. Although the very early steps in the postulated pathway have been studied and confirmed in vitro (Albro 1985), there is very little information available on the latter steps. Since the products of the first steps (2-ethylhexanol and MEHP) both produce toxic effects or effects that might be associated with toxicity (testicular atrophy and peroxisome proliferation in rodents), it is highly likely that the metabolism associated with toxicity occurs in the later steps of the postulated pathways. Indeed, it is in these later steps that the hard data are lacking.

Rats and mice differ in their metabolism of DEHP as reflected by the fact that the mouse excretory products are primarily in the form of

glucuronide conjugates while rats do not appear to excrete the products as conjugates. However, mouse urine contained all the metabolites found in rat urine plus glucuronides of most of them (Albro et al 1982). There are quantitative differences between the rat and mouse urinary metabolites. Most notable is the fact that metabolite V (2-ethyl-5-carboxypentylphthalate) exceeded by far all other metabolites in rat urine. Its presumed beta-oxidation product (2-ethyl-3-carboxypropylphthalate) predominated in mouse urine (Albro 1985). Thus, it is unlikely that the production of glucuronide conjugates in the mouse means that less oxidation takes place in the mouse cells. In addition, Dr. Albro stated that in vitro studies indicate that rats can form the glucuronide conjugate (personal presentation at the CHAP Meeting April 3-4, 1985). Apparently, it is degraded rather than excreted. When the extent of oxidation was estimated by measuring the amount of nicotinamide adenine dinucleotide (NAD) reduced, there was very little difference (2.93 moles of NAD vs. 2.23 moles of NAD) between the rat and mouse. However, there appears to be a significant difference in the extent of oxidation in rodents relative to the African green monkey (1.42 moles) and human leukemia patients (1.48 moles). While it is tempting to speculate that there is a direct relationship between reduced oxidation and the small amount of metabolite I in the primates, the connection is not supported by the rodent data. Although rats and mice show similar amounts of NAD reduced by DEHP, the difference in metabolite I production (16.8 mice vs. 9.4 rats) is in the wrong direction.

5. DEHP Metabolism and Peroxisome Proliferation

Although some elegant studies have been done on the metabolism of DEHP, the active intermediate that initiates carcinogenicity has not been identified and no cellular mechanism is firmly established. The possible mechanism that has received the most attention is that of peroxisome proliferation, an attractive hypothesis that needs further development. Nevertheless, it has not been established that a single metabolite or metabolic pathway is essential for peroxisome proliferation. Mitchell et al (1984) showed that MEHP, metabolite IX, or metabolite VI could induce peroxisome proliferation in cultured rat hepatocytes; while 2-ethylhexanol, metabolite V, or metabolite I did not. Since the W-I-oxidation pathway for DEHP metabolism has the sequence:

DEHP ⟶ MEHP ⟶ METABOLITE ⟶ METABOLITE VI

it is tempting to speculate that metabolite VI is the primary peroxisome inducer. However, an analysis of the data presented in further reports from this group (Lhugenot et al 1984) and others (Moody and Reddy 1978) suggests that this may not be the case. Mitchell et al (1984) showed that substantial peroxisome induction did not occur in cultured rat hepatocytes until the concentration of MEHP, metabolite IX, or metabolite VI was 500 uM. Lhuguenot et al (1984) showed that when the cultured rat hepatocytes were incubated with 500 uM MEHP for 3 days, the maximum concentrations of metabolites IX and VI were 69 uM and 74 uM respectively. Thus, it is unlikely that peroxisome induction, observed

when cultured rat hepatocytes were incubated with MEHP, can be accounted for by the conversion of MEHP to metabolites IX and VI.

Non-linearity in the dose dependent effects of DEHP on its metabolism has been observed (Robinson et al 1985). The non-linearity favors the excretion of increased proportions of metabolite I at higher doses of DEHP. Turnbull and Rodricks (1984) advanced the hypothesis that beta-oxidation of metabolite V, converting it to metabolite I, is the primary event leading to peroxisome proliferation. Although the metabolic pathways by which DEHP is converted to metabolite I have not been established, it is reasonable to assume that beta-oxidation of metabolite V, alpha-oxidation of metabolite V, and oxidation via mitochondrial respiration all play a role the formation of metabolite I. The relative contributions of these and other pathways to the formation of metabolite I have not been established.

The hypothesis that metabolite V conversion to metabolite I triggers peroxisome proliferation is not supported by the data of Mitchell et al (1984), who showed that MEHP, metabolite IX and metabolite VI induced peroxisomal activity in cultured hepatocytes but metabolites V and I did not.

It is clear from the above discussion that attempts to relate a specific metabolite or metabolic pathway to the triggering of peroxisomal proliferation have resulted in data that are in some cases conflicting and in other cases open to varied interpretation. With so many limitations, it would be premature to use species differences in metabolite profiles to predict species differences in susceptibility to cancer from exposure to DEHP.

Concluding Statements:

Although there are considerable data on the disposition of DEHP and its hydrolysis products, these are of little value, at present, in assessing the toxic potential for DEHP. There are no studies which compare the tissue levels of DEHP or its metabolic products with observed toxicity. In addition, it is not known which, if any, of the identified metabolites is/are responsible for observed toxicity.

F. Conclusions

1) Dermal absorption of DEHP has been shown to occur in rats.
2) The extent of dermal absorption is low (6.9% of the applied dose in 5 days).
3) The chemical identity of the dermally absorbed DEHP has not been established. Since skin possesses some DEHP hydrolase activity, conversion to MEHP may occur prior to or during absorption.
4) Data on the extent of DEHP dermal absorption by neonates (human or other species) are not available.
5) DEHP is readily absorbed after oral administration, but the absorbed species are primarily MEHP and EH.
6) Evidence has been reported for a threshold dose for absorption of intact DEHP following oral administration. However, in some studies using a different sex and strain of rats or strain of mice, evidence for a threshold dose was not found.
7) Some minor species and sex differences in the activity of nonspecific lipases towards DEHP have been shown.
8) Species differences in DEHP metabolism appear to be quantitative rather than qualitative.
9) Data on neonatal DEHP hydrolysis are not available, but it has been

found that senescent rats and adult rats appear to have equivalent activity.

10) Following continuous feeding of DEHP, intact DEHP has been found in at least four tissues including the liver.

11) As the orally administered dose increased, the amount of DEHP equivalents in the tissues increased.

12) DEHP equivalents have been shown to pass through the placenta but there was no specific accumulation of DEHP equivalents in the fetal side relative to the maternal side.

13) DEHP would be expected to pass the oral mucosa since it has a relatively high oil/water partition coefficient and the oral mucosa is a continuously hydrated surface (which increases pore size).

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