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July 15, 2004

Dr. C. W. Jameson
National Toxicology Program
Report on Carcinogens
79 Alexander Drive, Building 4401, Room 3118
P.O. Box 12233
Research Triangle Park, NC 27709

JUL 16 2004

RE: 12th Report on Carcinogens
Nomination of DEHP for Delisting

Dear Dr. Jameson:

The American Chemistry Council Phthalate Esters Panel (Panel) submits these materials in response to the National Toxicology Program call for public comment on substances proposed for listing or delisting in the 12th Report on Carcinogens (RoC). 69 Fed. Reg. 28940 (May 19, 2004). The Panel's comments pertain to the request to delist di(2-ethylhexyl) phthalate (DEHP) from the RoC. The Panel consists of the major domestic producers and some users of phthalate esters, including DEHP.

In accordance with the conclusions of the International Agency for Research (IARC) on Cancer and other governmental and independent scientific bodies, the Panel strongly supports the request to delist DEHP from the RoC. The weight of the evidence demonstrates that the liver tumors in rodents, which were the basis for classifying DEHP as reasonably anticipated to cause cancer, are not relevant for human risk assessment. The rationale for this conclusion is set out in the IARC monograph and other publications. The Panel is submitting additional materials that illuminate the species differences in response to DEHP.

If you have any questions, please call Marian K. Stanley, Senior Director and Manager of the Phthalate Esters Panel, at (703) 741-5623, email her at [Marian_St Stanley@americanchemistry .com](mailto:Marian_St Stanley@americanchemistry.com), or write her at the address at the bottom of this letter.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Courtney M. Price". The signature is fluid and cursive, written over a white background.



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EXECUTIVE SUMMARY

The American Chemistry Council Phthalate Esters Panel (Panel) submits these materials in response to the National Toxicology Program (NTP) call for public comment on substances proposed for listing or delisting in the 12th Report on Carcinogens (RoC). 69 Fed. Reg. 28940 (May 19, 2004). The Panel's comments pertain to the request to delist di(2-ethylhexyl) phthalate (DEHP) from the RoC. The Panel consists of the major domestic producers and some users of phthalate esters, including DEHP.

The Panel strongly supports the request to remove DEHP from the RoC. The results of several recent reviews of the DEHP data base support a conclusion that DEHP cannot be reasonably anticipated to cause cancer in humans. These comments briefly describe several of the important recent reviews. NTP is urged to review those reports, both to understand the basis for the conclusions and to access listings of the relevant data.

The Panel's comments also provide information relevant to the consideration of DEHP's potential carcinogenicity in the following areas:

- Pharmacokinetic and metabolic differences that indicate humans are less susceptible to DEHP than are rodents;
- Information on the pharmacodynamics of the receptor PPAR α that suggests humans could be exposed to low levels of DEHP for long periods of time without increasing the likelihood of tumors;
- Studies indicating gene expression in the livers of animals that lead to peroxisome proliferation and other cellular events associated with hepatocarcinogenesis in rodents, and human cell data indicating humans do not respond to the same extent as rodents;
- The role of oncogenes in the carcinogenic process;
- The role of Kupffer cells in liver parenchymal cell proliferation;
- The lack of any epidemiological indication of increased cancer;
- Data showing that exposures to DEHP are extremely low.

TABLE OF CONTENTS

	<u>Page</u>
EXECUTIVE SUMMARY	i
INTRODUCTION	1
I. Several recent scientific reviews have concluded that DEHP is not likely to cause cancer in humans.....	2
II. Pharmacokinetics and metabolism data indicate primates are less susceptible to DEHP than rodents	3
III. Pharmacodynamics of PPAR α in tissues and its relation to carcinogenesis	4
IV. Gene expression and carcinogenesis.....	5
V. Role of oncogenes in carcinogenic process	7
VI. Kupffer cells and liver parenchymal cell proliferation	7
VII. There is no epidemiology data indicating an increased risk of cancer from DEHP exposure	8
VIII. Exposures of the general population to DEHP are extremely low	9
CONCLUSION.....	10
REFERENCES	11

INTRODUCTION

The American Chemistry Council Phthalate Esters Panel (Panel) submits these materials in response to the NTP call for public comment on substances proposed for listing or delisting in the 12th Report on Carcinogens (RoC). 69 Fed. Reg. 28940 (May 19, 2004). The Panel's comments pertain to the request to delist di(2-ethylhexyl) phthalate (DEHP) from the RoC. The Panel consists of the major domestic producers and some users of phthalate esters, including DEHP.¹

The Panel strongly supports the request to remove DEHP from the RoC. A strong consensus has been building in the scientific community that the tumors observed in rodents treated with DEHP are not relevant for human risk assessment. As discussed in Part I of these comments, this consensus is reflected in the decision of the International Agency for Research on Cancer (IARC) to reclassify DEHP as "not classifiable as to carcinogenicity to humans", in the conclusions of the recent International Life Sciences Institute work group on peroxisome proliferators, and in other reviews of the DEHP data base. The outcomes of these reviews indicate that DEHP cannot be reasonably anticipated to cause cancer in humans.

The remainder of these comments provides other information which is relevant to the issue of DEHP's potential carcinogenicity to humans. The information is in the following areas:

- Pharmacokinetic and metabolic differences between species that affect the carcinogenic potential of DEHP in humans (Part II);
- Implications of pharmacodynamics of the PPAR α receptor in tissues for carcinogenicity (Part III);
- Studies indicating gene expression in the livers of animals that lead to peroxisome proliferation and other cellular events associated with hepatocarcinogenesis in rodents (Part IV);
- The role of oncogenes in the carcinogenic process (Part V);
- The role of Kupffer cells in liver parenchymal cell proliferation (Part VI);
- The lack of any epidemiological indication of increased cancer (Part VII); and
- Data showing that exposures to DEHP are extremely low (Part VIII).

¹ The Panel members include: BASF Corporation, Eastman Chemical Company, ExxonMobil Chemical Company, Ferro Corporation, and Teknor Apex, Inc.

The Panel is providing with these comments some recent studies on DEHP pharmacokinetics. The Panel also will provide NTP with copies of any articles cited in these comments, upon request.

I. SEVERAL RECENT SCIENTIFIC REVIEWS HAVE CONCLUDED THAT DEHP IS NOT LIKELY TO CAUSE CANCER IN HUMANS

Since NTP initially listed DEHP as reasonably anticipated to be a human carcinogen, researchers have generated an extensive data set on peroxisome proliferators in general and on DEHP in particular. In general, the conclusions of these reviews have been to discount the potential relevance to humans of the tumors observed in rodents treated with DEHP. Important recent reviews are briefly described below. The NTP is encouraged to review those reports, not only to understand what led those scientists to their conclusions, but to access lists of data relevant to the topic.

- *IARC Classification.* NTP's original listing of DEHP as reasonably anticipated to cause cancer was based in part on the decision of the International Agency for Research on Cancer (IARC) to classify DEHP as a probable human carcinogen (Group 2). In 2000, IARC evaluated the body of data generated since its original classification and determined that DEHP should be reclassified as Group 3 – not classifiable as to carcinogenicity to humans, on the basis that “the mechanism by which di(2-ethylhexyl) phthalate increases the incidence of hepatocellular tumours in rats and mice is not relevant to humans” (IARC, 2000). The IARC summary for DEHP was provided to NTP with the Aekyung Petrochemical Co. nomination letter. The Panel assumes that NTP is already in possession of the full IARC monograph on DEHP, but would be pleased to provide a copy on request.
- *ILSI Workshop.* The International Life Sciences Institute (ILSI) Risk Science Institute formed a workgroup in 2001 to review information on the mechanisms by which peroxisome proliferating chemicals produce carcinogenic responses in rats and mice. The report of the workgroup was published in late 2003 (Klaunig et al., 2003). For peroxisome proliferators in general, the workgroup concluded: “In summary, the weight of evidence overall currently suggests that the rodent [mode of action] for liver tumors is not likely to occur in humans, taking kinetic and dynamic factors into account” (Klaunig et al., 2003, p. 693).² DEHP was included as a case study by the group, with the following outcome: “The data lead to a conclusion that a carcinogenic response induced via the [modes of action] for liver tumorigenesis in the rodent is not likely to occur in humans following exposure to DEHP” (Klaunig et al., 2003, p. 704). The Klaunig et al. paper can

² On the basis of the ILSI workgroup conclusions, the U.S. Environmental Protection Agency (EPA) has proposed a science policy: “When liver tumors are observed in long term studies in rats and mice, and 1) the data are sufficient to establish that the liver tumors are a result of a PPAR α agonist MOA and 2) other potential MOAs have been evaluated and found not operative, the evidence of liver tumor formation in rodents should not be used to characterize potential human hazard” (EPA, 2003, p. 15).

be accessed at:

<http://www.epa.gov/oscpmont/sap/2003/december9/crittox33665578003.pdf>

- *Health Canada Assessment.* As part of an evaluation of the use of DEHP in vinyl medical devices, Health Canada reviewed the cancer data and accepted the conclusions of IARC (2000) that DEHP is not classifiable as to its carcinogenicity to humans (Health Canada, 2002). The portions of the Health Canada assessment that discuss carcinogenicity were provided to NTP with the Aekyung Petrochemical Co. nomination letter. The full assessment can be accessed at: http://www.hc-sc.gc.ca/hpb-dgps/therapeut/htmleng/advcomm_eapdehp.html.³
- *Doull et al. Assessment.* In 1998, a panel of scientific experts, chaired by Dr. John Doull, reviewed the data for DEHP in light of EPA's draft cancer risk assessment guidelines. The panel concluded: "DEHP should be classified as unlikely to be a human carcinogen under any known conditions of human exposure" (Doull et al., 1999, p. 352).

Thus, the consensus of a large number of scientific experts is that DEHP is not reasonably anticipated to be a human carcinogen.

II. PHARMACOKINETICS AND METABOLISM DATA INDICATE PRIMATES ARE LESS SUSCEPTIBLE TO DEHP THAN RODENTS

The first step in metabolism of DEHP, and in activation of PPAR α leading to peroxisome proliferation and hepatocarcinogenesis, is the hydrolysis of one alcohol from the diester (Klaunig et al., 2003). While this step can be catalyzed by any number of enzymes, pancreatic lipases are likely responsible for this step in the gastrointestinal tract. Comparisons of enzyme activities of lipases from rodents, humans, and non-human primates indicate that the maximum reaction velocity (V_{max}) for this reaction in higher organisms is not as great as the V_{max} in rodents (Lake et al., 1977; Nachlas and Seligman, 1949; see Klaunig et al., 2003; Ito et al., 2003). Rodents convert DEHP to its corresponding monoester (monoethylhexyl phthalate, MEHP) which is then efficiently absorbed over a wide range of exposure levels (e.g., Albro et al., 1982; Albro and Lavenhar, 1989). Across species, however, there are differences in the rate of formation and absorption of MEHP following ingestion of DEHP. Nachlas and Seligman (1949) showed that pancreatic esterase and lipase from humans has slightly lower hydrolytic activity than enzymes from rat pancreas, depending on the substrate. In addition, Lake et al. (1977) showed that hydrolysis of DEHP in human intestinal mucosa was roughly 10 times slower than in intestinal mucosa from rats. Studies with human volunteers showed that even at very low exposure levels, DEHP is absorbed much less efficiently than it is in rodents (Anderson et al., 2001a).

³ The U.S. Food and Drug Administration (FDA) also evaluated the use of DEHP in vinyl medical devices (FDA, 2001). FDA did not set a cancer-based tolerable intake level for DEHP because "there is considerable uncertainty with regard to the carcinogenic potential of DEHP in humans" (FDA, 2001, p. 21). The FDA assessment can be viewed at <http://www.fda.gov/cdrh/ost/dehp-pvc.pdf>.

This difference in hydrolysis plays a major role in the difference in pharmacokinetics between non-human primates (marmosets) and rodents. Enclosed is a recent publication by Kessler et al. (2004) that shows blood levels of DEHP and MEHP in pregnant and non-pregnant rats and marmosets exposed to 30 and 500 mg/kg DEHP by gavage. The data show that levels of the active metabolite, MEHP, are higher in rodents compared with marmosets, especially at dose levels that produce an increase in liver tumors in rodents (David et al., 1999). Furthermore, Laignelet and Lhuguenot (2000a,b) showed the comparative pharmacokinetics between rats and mice which demonstrate that peak blood levels (C_{max}) are higher in mice than rats administered comparable dose levels. The peak blood level in mice receiving a single dose of 200 mg/kg DEHP was 91 nmol DEHP equivalents/g blood, while the peak level in rats was 58 nmol DEHP equivalents/g blood. Peak levels of MEHP were 84 nmol DEHP equivalents/g blood in mice compared with 36.4 nmol DEHP equivalents/g blood in rats. These data provide evidence that the increased sensitivity of mice to tumors can, at least in part, be explained by pharmacokinetic differences. Coupling this difference with the data that pharmacokinetic differences play a role in distinguishing the effects in rodents and primates, the conclusion is that primates are less susceptible than rodents for cancer based on metabolism and absorption.

Data from Astill (1989) support the conclusion that differences in esterase activity exist between rodents and primates. Astill treated cynomolgus monkeys with DEHP and compared the excretion with that of rodents. That data suggest that hydrolysis of DEHP was lower for the primate than for the rat. Thus, differences in the ability to hydrolyze the diester to the monoester suggest the differences in absorption could occur between humans and rodents. Pharmacokinetic studies in primates and humans have demonstrated that DEHP is absorbed from the GI tract, but Rhodes et al. (1986) showed the bioavailability of DEHP in marmoset monkeys is less than in rats. Across a wide dose range, rats absorbed at least 50% of orally administered DEHP as monoester and excreted it in the urine (Rhodes et al., 1986). In contrast, marmosets absorbed a much smaller fraction of high doses of orally administered DEHP. In a more recent study, as phthalate doses ranged from 30 to 500 mg/kg/day, absorption by rats was greater than that in marmosets, with the differences being approximately 2-3 fold based on peak blood levels, and approximately 7 fold based on the area under the curve (Kessler et al., 2004). Other studies demonstrated that primates excrete phthalates in the bile to a much greater extent than do rodents, and, therefore, much of what is absorbed in primates may not be available to the target organs (Kessler et al., 2004).

III. PHARMACOKINETICS OF PPAR α IN TISSUES AND ITS RELATION TO CARCINOGENESIS

Pharmacodynamic differences between species also play a role in the carcinogenic responses and should be considered by NTP. Human PPAR α receptors are not as sensitive to MEHP as are rodent receptors (Maloney and Waxman, 1999; Yu et al., 2001; see Klaunig et al., 2003). The difference in response to similar concentrations of MEHP is up to 1.8. Thus, humans may be half as susceptible to activation of PPAR α receptor as rodents.

Lower activation may also lead to diminished response in tissues. Lack of sufficient response, based on marker enzymes, or lack of specific gene expression, may explain why non-rodent tissues do not develop tumors. Dose-response data for tumor response and marker enzyme response show that minimal levels of “peroxisome proliferation” may be needed for tumorigenesis (Ashby et al., 1994; Klaunig et al., 2003). Thus, the presence of PPAR α receptor alone is not sufficient for cancer.

This concept has implication for carcinogenic potential in humans. It is known that human tissues contain PPAR α (Mukherjee et al., 1994; Auboeuf et al., 1997; Palmer et al., 1998; Walgren et al., 2000; Wurch et al., 2002) and that these receptors can be activated by substances known to cause cancer in rodent liver (Mukherjee et al., 1994; Maloney and Waxman, 1999; Walgren et al., 2000; Wurch et al., 2002). However, as stated above, potency for activation of the receptor and the subsequent magnitude of the response have an impact on whether tumors develop in rodents and would be likely to have an impact on whether tumors develop in humans. This potency is discussed in general terms for some substances that interact with the receptor, and for DEHP in particular, by Klaunig et al. (2003). Carcinogenesis does not appear to occur in rodents until a critical level of biochemical/cellular activity occurs, suggesting that any species, including humans, could be exposed to low levels of DEHP for long periods of time without increasing the likelihood of tumors.

IV. GENE EXPRESSION AND CARCINOGENESIS

The mode of action for PPAR α -agonist hepatocarcinogenicity described by Klaunig et al. (2003), while relatively complete, lacked specific information on gene activation. Several publications have mapped gene up- and down-regulation following exposure of rodents to peroxisome proliferators including DEHP. The following table provides information on gene expression following exposure to DEHP or other peroxisome proliferators. Some studies quantitated expression by mRNA levels, whereas more recent studies used gene arrays.

Gene expression following treatment with known PPAR α agonists

Gene expressed	Substance exposed	Test system	Reference
<i>c-fos</i> <i>c-jun</i> , <i>jun-B</i> , <i>jun-D</i>	Wy-14,645 DEHP	BNL-CL2 cells NIH 3T3 cells	Ledwith et al., 1993
<i>c-fos</i> <i>c-jun</i> , <i>junB</i> , <i>egr-1</i> , NUP475, <i>fosB</i>	Wy-14,645 DEHP clofibrate ciprofibrate	BNL-CL2 cells ML-457 cells	Ledwith et al., 1996
TGF- β_1 IGFII/Man6P	Nafenopin methylclofenapate Wy-14,643 clofibric acid	Rats	Rumsby et al., 1994
apo E, HD	DEHP Wy-14,643 clofibrate	Mice	Motojima, 1997
<i>c-fos</i> , <i>c-jun</i> , <i>c-myc</i>	DEHP	Rats	Hasmall et al., 1997
ACO, FABP, CYP4A1, <i>c-myc</i>	Wy-14,643	Rats	Belury et al., 1998
apo CIII, FACO, HD, THIO, CYP4A, PMP-70	Fenofibrate	HepG2 cells	Lawrence et al., 2001
U74Av2 Genechip	Wy-14,643 fenofibrate	Mice	Yamazaki et al., 2002
U74Av2 Genechip	DEHP	Mice	Wong and Gill, 2002
Custom Genechip ACO, ERK3, JAK1, JAK2, MKP-1, GSK3 α , GSK3 β	Wy-14,643	HepG2 cells FaO cells	Vanden Heuvel et al., 2003

Genes consistently up-regulated are associated with lipid homeostasis, steroid metabolism, or oxidative stress. Some detoxifying and proliferative genes are also up-regulated. No consistent pattern could be attributed to down-regulated genes. Interestingly, limited data from human cells do not show as many genes expressed in response to PPAR α -agonists as do rodent cells (Vanden Heuvel et al., 2003), which suggests that humans do not respond to the same extent as rodents to exposure from these substances.

V. ROLE OF ONCOGENES IN CARCINOGENIC PROCESS

For several studies listed above, oncogenes or proto-oncogenes were up-regulated following exposure to DEHP (Ledwith et al., 1993; Ledwith et al., 1996; Hassmall et al., 1997). The role of these genes in the MOA is important to understand so as not to confuse these oncogenes with other MOAs. It is believed that these genes result in an early mitogenic response in hepatocytes observed *in vivo* (Conway et al., 1989; David et al., 1999; James et al., 1998; Smith-Oliver and Butterworth, 1987). Indeed, transgenic mice carrying the human cHA-*ras* gene have shown tumorigenic responses after exposure to DEHP (Toyosawa et al., 2001), although not consistently (Freeman et al., 2000). The role of the oncogene in the tumorigenic response was minimal (Toyosawa et al., 2003). Others have proposed that hypomethylation of some proto-oncogenes may also be involved in the carcinogenic process (Ge et al., 2001; Ge et al., 2002). It is not clear how this hypomethylation step contributes to or detracts from the current MOA; however, it is not inconsistent with oncogene up-regulation leading to the mitogenic response.

VI. KUPFFER CELLS AND LIVER PARENCHYMAL CELL PROLIFERATION

The activation of Kupffer cells via a PPAR α -independent mechanism (Peters et al., 2000; Rusyn et al., 2001) by peroxisome proliferators (PPs) results in the release of TNF α (Bojes et al., 1997) and other potential mitogenic cytokines (Anderson et al., 2001b). This observation has led to the proposal that Kupffer cell activation, not PPAR α activation of hepatic parenchymal cells, may be causally responsible for the mitogenic effects of peroxisomal proliferators in the rodent liver (Rose et al., 1997a). However, a review of the available data indicates that while Kupffer cell activation can augment DNA proliferative responses of hepatocytes to PPs, the hepatocellular tumors seen in rodents chronically exposed to PPs are ultimately dependent on the presence of PPAR α positive hepatocytes.

Support for this contention comes from several lines of investigation:

- Kupffer cells are a rich source of mitogens such as TNF α . TNF α , but not the potent PP Wy-14,643, increases DNA synthesis in isolated rat hepatocytes. However, when exposure to TNF α and Wy-16,463 are combined, the increase in DNA synthesis is significantly greater than that seen with TNF α alone (Parzefall et al., 2001).

- The increased hepatocellular proliferation induced by Wy-14,643 can be blocked by antibodies to TNF α (Bojes et al., 1997). However, TNF α signaling is not required for Wy-14,643 induced hepatocyte proliferation because mice nullizygous for TNF-receptor 1 and/or TNF-receptor 2 gave proliferative responses identical to those of wild-type mice (Anderson et al., 2001b).
- Addition of Kupffer cells from either wild-type or PPAR α -null mice to isolated mouse hepatocytes restores the ability of the PP nafenopin to increase DNA synthesis in wild-type hepatocytes but not PPAR α -null hepatocytes (Hasmall et al., 2001)
- Inactivation of Kupffer cells *in vivo* with methyl palmitate (Rose et al., 1997a) or glycine (Rose et al., 1997b) blocks PP-induced hepatocyte proliferation at 24-hr and 3-weeks post-exposure but not at 22-weeks post-exposure (Rose et al., 1999). At 51-weeks post-exposure, rats exposed to PP plus glycine exhibited fewer hepatic tumors than rats exposed to PP alone (Rose et al., 1999). The authors hypothesized that glycine inhibited tumor growth by preventing angiogenesis.
- The chronic administration of Wy-14,643 to wild-type and PPAR α -null mice resulted in increased hepatocellular proliferation at 1 and 5 weeks as well as multiple hepatocellular neoplasms at 11 months in only the wild-type mice; PPAR α -null mice were unaffected (Peters et al., 1997).
- The requirement for PPAR α in PP-induced proliferative and neoplastic responses received further support from a recent study in which chimeric livers containing either PPAR α -null or wild-type donor hepatocytes and either PPAR α -null or wild-type host hepatocytes were exposed to Wy-14,643 (Weglarz and Sandgren, 2004). In this study, both PPAR α -null or wild-type hepatocytes in the chimeric liver exhibited elevated DNA synthesis as long as some of the hepatocytes (i.e., donor or host) contained PPAR α .

VII. THERE IS NO EPIDEMIOLOGY DATA INDICATING AN INCREASED RISK OF CANCER FROM DEHP EXPOSURE

IARC determined that adequate epidemiologic studies of workers exposed to DEHP have not been conducted (IARC, 2000). Limited studies are available and were reviewed by Klaunig et al. (2003). Thiess et al. (1978) reported on a small study involving 101 employees exposed to DEHP during production. Airborne concentrations of DEHP were 0.003–0.004 ppm. The subjects ranged in age from 22 to 60 years of age and had been exposed for months to 35 years (average of 12 years). There were 8 deaths among this group, compared with 15.9 expected deaths in the geographic area and 17.0 expected from the national rate. There was one death from pancreatic cancer (with 0.13 expected) and one bladder papilloma (0.01 expected) in workers with the longest employment. Workers in the PVC industry have also been evaluated. Hagmar et al. (1990), Hardell et al. (1997), and Ohlson and Hardell, (2000) reported on the incidence of

cancer in workers in the PVC industry. No conclusions can be drawn because DEHP exposures were not provided, and exposure to other chemicals cannot be discounted.

VIII. EXPOSURES OF THE GENERAL POPULATION TO DEHP ARE EXTREMELY LOW

For several years, the Centers for Disease Control and Prevention (CDC) have been analyzing phthalate metabolite (monoester) levels in urine samples from the U.S. population. Those results can be used to calculate the exposure to the parent phthalate (Kohn et al., 2000; David, 2000).⁴ The most recent report of CDC's biomonitoring is the Second National Report on Human Exposure to Environment Chemicals (CDC, 2003), which provides a summary of phthalate metabolite measurements for approximately 2500 persons, a statistically representative sample of the U.S. population. When converted to parent exposures,⁵ those data indicate that the average exposure to DEHP is 0.61 ug/kg/day and the 95th percentile is 3.5 ug/kg/day.

Koch et al. (2003) analyzed samples from 85 subjects for different DEHP metabolites than the monoester studied by the CDC. They calculated the average exposure to be 13.8 ug/kg/day, with a 95th percentile value of 52.1 ug/kg/day. However, the molar conversion factors they used were taken from a relatively old and limited study -- Schmid and Schlatter (1985). Koch et al. (2004) have more recently reported that the molar conversion factors for MEHP and its secondary metabolites are approximately a factor of 3 higher than those reported by Schmid and Schlatter. Since the molar conversion factor is in the denominator of the exposure calculation, these data suggest that Koch and associates may have overestimated human exposure to DEHP.

The reviews of DEHP are in agreement that it is not genotoxic. The conclusions of the various reviews that DEHP does not pose a carcinogenic risk to humans are based on species differences in the response to DEHP and species differences in DEHP pharmacokinetics. The very low exposures of the general population to DEHP shown by the CDC data provide further reassurance that it cannot be reasonably anticipated to cause cancer in humans.

⁴ Note that the conversion equation in the David article was incorrectly formatted. The correct equation is as follows:

$$\text{daily intake } (\mu\text{g/kg/day}) = \left(\frac{\text{urine conc. } (\mu\text{g/g creatinine}) \times \text{creatinine excretion } (\text{g/kg/day}) \times \text{mol wt diester } (\text{g/mol})}{\text{mol w. monoester } (\text{g/mol}) \times \left(\frac{\text{mol monoester in urine}}{\text{mol diester ingested}} \right)} \right)$$

⁵ Conversion done using the methodology of David (2000). The values used for creatinine excretion were taken from Tietz (1986), p. 1821. Values for the ratio of monoester in urine to diester ingested were taken from Anderson et al. (2001a).

CONCLUSION

The results of several recent reviews of the DEHP data base support a conclusion that DEHP cannot be reasonably anticipated to cause cancer in humans. The Panel therefore supports the request to delist DEHP from the Report on Carcinogens. Additional information provided in these comments should be useful to NTP as it evaluates the potential carcinogenicity of DEHP.

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**DI-(2-ETHYLHEXYL)PHTHALATE (DEHP)
ABSORPTION, EXCRETION, METABOLISM AND
PHARMACOKINETIC PROFILE IN FEMALE CD-1 MICE**

REPORT no. 4/99

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I have reviewed this report and concur with its content.

Jean-Claude Lhuguenot, PhD

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Date 22.12.2000

I, the undersigned, was responsible for the conduct of the work and reporting of the results. I concur with the views expressed in the report.

Laurence Laignelet, PhD

(Study director)



Date 22.12.2000

CONTENTS

Title page	
Signature page	1
1. SUMMARY	8
2. INTRODUCTION	10
3. MATERIALS	12
3.1 Test Material	12
3.1.1 Non-radioactive test material	12
3.1.2 Radiolabelled test material	12
3.2 Reagents and chemical	12
3.3 Animals	12
3.4 Instrumentation	12
4. METHOD	13
4.1 Animals	13
4.1.1 Identification	13
4.1.2 Acclimatisation	13
4.1.3 Environmental control	13
4.1.4 Animal accommodation	13
4.1.5 Cage identification	13
4.1.6 Diet and water supply	13
4.2 Determination of radioactivity	13
4.2.1 Background radioactivity	14
4.2.2 Limit of detection	14
4.3 Gas chromatography analysis	14
4.3.1 Gas chromatography conditions	14
4.3.2 Quantification	14
4.3.3 MEHP-derived metabolites identification	14
4.4 Dose formulation	16
4.5 Administration	16
4.6 Treatment groups	16
4.6.1 Blood radioactivity	16
4.6.1.1 Single oral dose	16
4.6.1.2 Repeated oral dose	17
4.6.2 Mass balance excretion study	17
4.6.2.1 Single oral dose	17
4.6.2.2 Repeated oral dose	17
4.7 Body weights	17
4.8 Sampling and storage	17
4.8.1 Identification	17
4.8.2 Urine and faeces	17
4.8.3 Blood and plasma	18
4.9 Sample analysis	18
4.9.1 Radioactivity analysis	18
4.9.1.1 Dose solution	18
4.9.1.2 Urine	18
4.9.1.3 Faeces	18
4.9.1.4 Blood	18
4.9.2 Gas chromatography analysis	19
4.9.2.1 Urine	19
4.9.2.2 Faeces	19
4.9.2.3 Preparation of derivatives	19
4.10 Pharmacokinetic data analysis	19

5. RESULTS	20
5.1 Pharmacokinetic study	20
5.1.1 Single dosing	20
5.1.1.1 Body weights	20
5.1.1.2 Recovery of radioactivity	20
5.1.1.2.1 Dose level 200 mg/kg	20
5.1.1.2.2 Dose level 1000 mg/kg	20
5.1.2 Repeated dosing	20
5.1.2.1 Body weights	20
5.1.2.2 Recovery of radioactivity	20
5.1.2.2.1 Dose level 200 mg/kg	20
5.1.2.2.2 Dose level 1000 mg/kg	21
5.2 Excretion and metabolism study	21
5.2.1 Single dosing	21
5.2.1.1 Body weights	21
5.2.1.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces.....	21
5.2.1.2.1 Recovery of radioactivity	21
5.2.1.2.1.1 Dose level 200 mg/kg	21
5.2.1.2.1.2 Dose level 1000 mg/kg	21
5.2.1.2.2 Analysis by gas chromatography	22
5.2.1.2.2.1 Dose level 200 mg/kg	22
5.2.1.2.2.2 Dose level 1000 mg/kg	22
5.2.2 Repeated dosing	23
5.2.2.1 Body weights	23
5.2.2.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces.....	24
5.2.2.2.1 Recovery of radioactivity	24
5.2.2.2.1.1 Dose level 200 mg/kg	24
5.2.2.2.1.2 Dose level 1000 mg/kg	24
5.2.2.2.2 Analysis by gas chromatography	25
5.2.2.2.2.1 Dose level 200 mg/kg	25
5.2.2.2.2.2 Dose level 1000 mg/kg	27
6. DISCUSSION	29
7. REFERENCES	31
FIGURES	32
Pharmacokinetic study	32
Figure 1. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice following a single oral administration of [¹⁴ C]-DEHP at 200 or 1000 mg/kg.	32
Figure 2. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice following a single oral administration of 200 or 1000 mg/kg [¹⁴ C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled-DEHP, respectively.	33
Excretion and metabolism study, single dosing	34
Figure 3. Recovery of radioactivity (in % of the DEHP dose) in urine and faeces from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg [¹⁴ C]-DEHP.	34
Figure 4. Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	35
Figure 5. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	36
Figure 6. Distribution of free and conjugate metabolites in urine from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	36
Figure 7. Cumulative excretion (0-72h) and distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in µmoles) in urine from female mice following a single oral administration of 200 mg/kg DEHP.	38

Figure 8. Cumulative excretion (0-72h) and distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice following a single oral administration of 1000 mg/kg DEHP.	39
Figure 9. Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	40
Figure 10. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	41
Figure 11. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in the urine and faeces of female mice treated with a single oral dose of 200 mg/kg or 1000 mg/kg DEHP.	42
Excretion and metabolism study, repeated dosing.	43
Figure 12. Mean recovery of radioactivity (in μ moles DEHP-equivalents) in urine and faeces from female mice collected within 24 hours following the 1 st (on D0) to the 9 th (on D8 included) oral administration of 200 mg/kg/d [¹⁴ C]-DEHP.	43
Figure 13. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female mice collected within 24 hours following the 1 st (on D0) to the 9 th (on D8 included) oral administration of 200 mg/kg/d [¹⁴ C]-DEHP.	44
Figure 14. Mean recovery of radioactivity (in μ moles) in urine and faeces from female mice collected within 24 hours following the 1 st (on D0) to the 9 th (on D8 included) oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP.	45
Figure 15. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female mice collected within 24 hours following the 1 st (on D0) to the 9 th (on D8 included) oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP.	46
Figure 16. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice within 24 hours following the 1 st and 4 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.	47
Figure 17. Distribution of free and conjugate metabolites in urine of female mice within 24 hours following the 1 st and 4 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.	48
Figure 18. Distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice within 24 hours following the 1 st and 4 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.	49
Figure 19. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female mice within 24h hours following the 1 st , 4 th and 7 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.	50
Figure 20. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in the urine and faeces of female mice within 24 hours following the 1 st and 4 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.	51
Figure 21. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice within 24 hours following the 1 st , 4 th and 7 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.	52
Figure 22. Distribution of free and conjugate metabolites in urine of female mice within 24 hours following the 1 st , 4 th and 7 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.	53
Figure 23. Distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice within 24 hours following the 1 st , 4 th and 7 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.	54
Figure 24. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female mice within 24 hours following the 1 st , 4 th and 7 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.	55
Figure 25. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in the urine and faeces of female mice within 24 hours following the 1 st , 4 th and 7 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.	56
TABLES	57
Pharmacokinetic study	57
Table 1. Mean (and standard deviation) body weight values in female mice treated with a single administration of 200 or 1000 mg/kg DEHP on Day 0.	57
Table 2. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice following a single oral administration of 200 or 1000 mg/kg [¹⁴ C]-DEHP.	57

Table 3. Mean (and standard deviation) body weight values in female mice treated with a 6-day repeated oral administration of 200 or 1000 mg/kg DEHP from D0 to D5 (inclusive).....	58
Table 4. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice after a single administration 200 or 1000 mg/kg of [¹⁴ C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled DEHP, respectively.....	59
Excretion and metabolism study, single dosing.....	60
Table 5. Mean (and standard deviation) body weight values in control female mice and in female mice treated with a single administration of 200 or 1000 mg/kg [¹⁴ C]-DEHP on Day 0.....	60
Table 6. Recovery of radioactivity (as percentage of the [¹⁴ C]-DEHP dose and as μmole DEHP-equivalents) in urine and faeces from female mice following a single oral administration of 200 mg/kg.....	61
Table 7. Recovery of radioactivity (as percentage of the [¹⁴ C]-DEHP dose and as μmole DEHP-equivalents) in urine and faeces from female mice following a single oral administration of 1000 mg/kg.....	61
Table 8. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (expressed as μmoles or as % of the DEHP dose) excreted in urine from female mice following a single oral administration of 200 mg/kg.....	62
Table 9. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (expressed as μmoles or as % of the DEHP dose) excreted in faeces from female mice following a single oral administration of 200 mg/kg.....	64
Table 10. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (expressed as μmoles or as % of the DEHP dose) excreted in urine from female mice following a single oral administration of 1000 mg/kg.....	65
Table 11. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (expressed as μmoles or as % of the DEHP dose) excreted in faeces from female mice following a single oral administration of 1000 mg/kg.....	66
Excretion and metabolism study, repeated dosing.....	68
Table 12. Mean (and standard deviation) body weight values in control female mice and in female mice treated with a 9-day repeated oral administration (fromn D0 to D8 inclusive) of 200 or 1000 mg/kg/d [¹⁴ C]-DEHP.....	68
Table 13. Mean recovery of radioactivity in urine from female mice collected within 24 hours following the 1 st to the 9 th oral administration of 200 mg/kg/d [¹⁴ C]-DEHP.....	69
Table 14. Mean recovery of radioactivity in faeces from female mice collected within 24 hours following the 1 st to the 9 th oral administration of 200 mg/kg/d [¹⁴ C]-DEHP.....	70
Table 15. Mean recovery of radioactivity in urine and faeces from female mice collected within 24 hours following the 1 st to the 9 th oral administration of 200 mg/kg/d [¹⁴ C]-DEHP.....	71
Table 16. Mean recovery of radioactivity in urine from female mice collected within 24 hours following the 1 st to the 9 th oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP.....	72
Table 17. Mean recovery of radioactivity in faeces from female mice collected within 24 hours following the 1 st to the 9 th oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP.....	73
Table 18. Mean recovery of radioactivity in urine and faeces from female mice collected within 24 hours following the 1 st to the 9 th oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP.....	74
Table 19. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female mice collected within 24 hours following the 1 st , 4 th and 7 th oral administration of 200 mg/kg/d.....	75
Table 20. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in faeces from female mice collected within 24 hours following the 1 st , 4 th and 7 th oral administration of 200 mg/kg/d.....	77
Table 21. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female mice collected within 24 hours following the 1 st , 4 th and 7 th oral administration of 1000 mg/kg/d.....	78
Table 22. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in faeces from female mice collected within 24 hours following the 1 st , 4 th and 7 th oral administration of 1000 mg/kg/d.....	80

APPENDIXES	81
Pharmacokinetic study, single dosing	81
Appendix 1. Individual body weight values for female mice treated with a single oral administration of 200 mg/kg (mice 1 to 6) or 1000 mg/kg (mice 7 to 12) of [¹⁴ C]-DEHP on day 0	81
Appendix 2. Recovery of radioactivity in blood (in dpm/g blood and nmole DEHP-equivalents/g blood) from female mice following a single oral administration of [¹⁴ C]-DEHP	82
Pharmacokinetic study, multiple dosing	83
Appendix 3. Individual body weight values for female mice treated with a 6-day repeated oral administration of 200 mg/kg/d DEHP from D0 to D5 (inclusive)	83
Appendix 4. Individual body weight values for female mice treated with a 6-day repeated oral administration of 1000 mg/kg/d DEHP from D0 to D5 (inclusive)	84
Appendix 5. Recovery of radioactivity in blood (in dpm/g blood and nmole DEHP-equivalents/g blood) from female mice following a single oral administration of [¹⁴ C]-DEHP which was preceded by a 5-day treatment with unlabelled DEHP	85
Excretion and metabolism study, single dosing	86
Appendix 6. Individual body weight values for control female mice (T1 to T4) and female mice treated with a single oral dose of 200 mg/kg (mice 1 to 6) or 1000 mg/kg (mice 7 to 12) [¹⁴ C]-DEHP	86
Appendix 7. Recovery of radioactivity (dpm) in the urine and faeces from female mice following a single oral administration of 200 mg/kg	87
Appendix 8. Recovery of radioactivity (dpm) in the urine and faeces from female mice following a single oral administration of 1000 mg/kg	88
Appendix 9. Calculated recovery of radioactivity (as % of the dose or μmole DEHP-equivalents) excreted in urine and faeces from female mice following a single oral administration of 200 mg/kg	89
Appendix 10. Calculated recovery of radioactivity (as % of the dose or μmole DEHP-equivalents) excreted in urine and faeces from female mice following a single oral administration of 1000 mg/kg	90
Appendix 11. Gas chromatography analysis of urine of female mice treated with a single oral dose of 200 mg/kg DEHP. Peak areas values and percentage of total for each metabolites	91
Appendix 12. Gas chromatography analysis of urine of female mice treated with a single oral dose of 1000 mg/kg DEHP. Peak areas values and percentage of total for each metabolites	94
Appendix 13. Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (as μmoles or % of the dose) in urine from female mice following a single oral administration of 200 mg/kg DEHP	97
Appendix 14. Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (as μmoles or % of the dose) in urine from female mice following a single oral administration of 1000 mg/kg DEHP	100
Appendix 15. Recovery of [¹⁴ C]-DEHP (as % of total radioactivity) in the faeces from female mice following a single administration of 200 mg/kg [¹⁴ C]-DEHP	103
Appendix 16. Recovery of [¹⁴ C]-DEHP (as % of total radioactivity) in the faeces from female mice following a single administration of 1000 mg/kg [¹⁴ C]-DEHP	104
Appendix 17. Gas chromatography analysis of faeces from female mice following a single oral administration of 200 mg/kg DEHP. Peak areas values and percentage of total for each metabolites	105
Appendix 18. Gas chromatography analysis of faeces from female mice following a single oral administration of 1000 mg/kg DEHP. Peak areas values and percentage of total for each metabolites	106
Appendix 19. Individual calculated amount of DEHP and MEHP-derived metabolites (as μmoles or as % of the dose) identified in faeces from female mice following a single oral administration of 200 mg/kg DEHP	107
Appendix 20. Individual calculated amount of DEHP and MEHP-derived metabolites (as μmoles or as % of the dose) identified in faeces from female mice following a single oral administration of 1000 mg/kg DEHP	108
Excretion and metabolism study, repeated dosing	109
Appendix 21. Individual body weight values for control female mice (T1 to T3) and female mice treated with a 9-day repeated oral administration of 200 mg/kg/d (mice 1	

to 6) or 1000 mg/kg/d (mice 7 to 12) of [¹⁴C]-DEHP from D0 to D8 (inclusive). 109

Appendix 22. Recovery of radioactivity (in dpm) in the urine and faeces from female mice collected within 24 hours following oral administration of 200 mg/kg/d from D0 to D8 (inclusive). 110

Appendix 23. Individual calculated amount of radioactivity in the urine and faeces from female mice collected within 24 hours following the oral administration of 200 mg/kg/d from D0 to D8 (inclusive). 112

Appendix 24. Individual recovery of radioactivity (in dpm) in the urine and faeces from female mice collected within 24 hours following the oral administration of 1000 mg/kg/d from D0 to D8 (inclusive). 114

Appendix 25. Individual calculated amount of radioactivity in the urine and faeces from female mice collected within 24 hours following the oral administration of 1000 mg/kg/d from D0 to D8 (inclusive). 116

Appendix 26. Gas chromatography analysis of urine from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 200 mg/kg/d [¹⁴C]-DEHP on day 0, 3 and 6. Peak areas values and percentage of total for DEHP, MEHP and MEHP-derived metabolites. 118

Appendix 27. Gas chromatography analysis of urine from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP on day 0, 3 and 6. Peak areas values and percentage of total for DEHP, MEHP and MEHP-derived metabolites. 120

Appendix 28. Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (in µmoles) present in urine from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 200 mg/kg/d of DEHP on day 0, 3 and 6. 122

Appendix 29. Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (in µmoles) present in urine from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg/d DEHP on day 0, 3 and 6. 124

Appendix 30. Recovery of [¹⁴C]-DEHP in the faeces from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 200 mg/kg/d (group A and B) or 1000 mg/kg/d (group C and D) of [¹⁴C]-DEHP on day 0, 3 and 6. 126

Appendix 31. Gas chromatography analysis of faeces from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 200 mg/kg/d [¹⁴C]-DEHP on day 0, 3 and 6. Peak areas values and percentage of total for DEHP, MEHP and MEHP-derived metabolites. 127

Appendix 32. Gas chromatography analysis of faeces from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP on day 0, 3 and 6. Peak areas values and percentage of total for DEHP, MEHP and MEHP-derived metabolites. 128

Appendix 33. Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (in µmoles) present in faeces from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 200 mg/kg/d on day 0, 3 and 6. 129

Appendix 34. Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (in µmoles) present in faeces from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg/d on day 0, 3 and 6. 130

1. SUMMARY

Di(2-ethylhexyl)phthalate (DEHP) is extensively used as plasticiser for polyvinyl chloride. The absorption, blood concentration and excretion of DEHP was determined in CD1 female mice following a single and a repeated oral administration at the dose levels of 200 mg/kg and 1000 mg/kg. Blood samples were taken at defined time intervals after administration for quantification of total radioactivity. Urine and faeces were collected daily and DEHP and its metabolites were extracted and then identified by GC-MS and quantified by GC.

The absorption of radioactive material following single oral doses of [^{14}C]-DEHP at 200 mg/kg and 1000 mg/kg to female CD-1 mice was rapid. The maximum blood concentrations (C_{max}), approximately 154 and 1339 nmole DEHP-equivalents/g for the low and the high doses, were obtained 0.5 hour and 1.5 hours after dosing, respectively. The half-life of elimination were approximately 7.1 and 10.3 hours at the low and the high dose level, respectively. The areas under the curves ($\text{AUC}_{0-48\text{h}}$) were 2069 and 6838 nmole DEHP-equivalents.h.g $^{-1}$ for 200 mg/kg and 1000 mg/kg dose levels, respectively. The 8.7-fold difference in the C_{max} between dose levels and the 3.3-fold difference in AUC are at variance with the 5-fold difference in dose levels. Negligible or low levels of radioactivity (approximately 1% of the C_{max}) were detected in blood samples 48 hours for the low and the high doses, respectively.

The absorption of the radioactive material was rapid, following a single dose of 200 or 1000 mg/kg [^{14}C]-DEHP administered after a 5-day pre-treatment of female CD-1 mice with 200 or 1000 mg/kg bw/d of unlabelled DEHP. The C_{max} , approximately 197 and 396 nmole-DEHP equivalents/g for the low and the high doses, were obtained 1.5 hours and 4 hours after dosing, respectively. The half-life of elimination were approximately 7.1 and 7.9 hours at the low and the high dose level, respectively. The $\text{AUC}_{0-48\text{h}}$ were 2252 and 5672 nmole DEHP-equivalents.h.g $^{-1}$ for 200 and 1000 mg/kg dose levels, respectively. This 2-fold difference in the C_{max} between dose levels and the 2.5-fold difference in AUC is at variance the 5-fold difference in dose levels and can indicate a saturation of the absorption process. Negligible or low level of radioactivity (approximately 1-1.5% of the C_{max}) were detected in blood samples at 48 hours for the low and the high doses, respectively.

According to the previous results, a 5-day pre-treatment with unlabelled DEHP at 200 mg/kg bw/d induced a 28% and 9% increase of the C_{max} and the AUC of the radioactive dose, respectively. In contrast, at 1000 mg/kg bw/d, a 5-day pre-treatment induced a 3.4-fold decrease of the C_{max} and 17% decrease of the AUC.

After a single oral administration to female CD-1 mice, excretion of radioactive material was very rapid as most was excreted in the first 48 hours. Following the low and high dose administrations, 44.4% and 31.0% of the dose were recovered in the urine and 15.4% and 28.2% in the faeces within 96 hours, respectively. The total overall mean recovery was approximately 60% at both dose levels.

Approximately 4.4 and 18.3% of the low and high DEHP dose were excreted unchanged almost totally in faeces, either due to a non absorption or/and a re-excretion as a consequence of an entero-hepatic recirculation. Approximately 13.4 and 12.3% of the low and high doses were excreted as MEHP, the proximate metabolite issued from DEHP hydrolysis, respectively. MEHP was excreted approximately 50/50 in urine and faeces at both dose levels. Total excretion of MEHP-derived metabolites reached 40 and 26% of the dose at 200 mg/kg and 1000 mg/kg, respectively. They were essentially excreted in urine (35% and 22% of the low and high DEHP dose, respectively) and for a small part in faeces (4.1% and 4% of the low and high DEHP dose, respectively). At 200 and 1000 mg/kg, approximately 58 and 49% of MEHP, and 54 and 13% of MEHP-derived metabolites were excreted in urine as glucuro-conjugates, respectively. Whatever the dose level, MEHP ω -1 oxidation was the main metabolic pathway (about 70%), followed by ω -oxidation (about 30%).

During a 9-day repeated administration at 200 and 1000 mg/kg bw/d [^{14}C]-DEHP to female mice, approximately 36.2 and 36.7.8% of the total [^{14}C]-DEHP doses were recovered in the urine and 32.0 and 32.7% in the faeces, respectively. At both dose levels, daily excretion was stable with mean values of 5.9 ± 0.9 and 26.5 ± 7.5 $\mu\text{mole DEHP-equivalents/day}$ in urine and 4.8 ± 1.2 and 24.9 ± 8.8 $\mu\text{mole DEHP-equivalents/day}$ in faeces, respectively. The total overall mean recovery of radioactive material in excreta was approximately 68% at 200 mg/kg bw/d and 69% at 1000 mg/kg bw/d. These values of total excretion

and the observed steady-state excluded any significant bio-accumulation of DEHP.

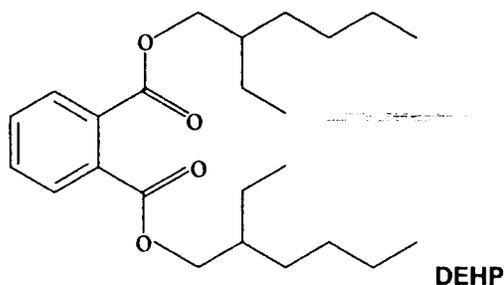
During the repeated administration of 200 mg/kg bw/d DEHP for 9 days to female CD-1 mice, about 13 and 23% of the DEHP daily dose were excreted almost totally in faeces (97-98%) as non-metabolised DEHP within 24 hours after the 1st and 4th administration, representing 26 and 37% of the total daily recovery in excreta, respectively. Only trace amounts of non-metabolised DEHP were detected in urine. Within 24 hours after the 1st and 4th administration, about 11 and 15% of the DEHP daily dose were excreted as MEHP mainly in urine (71 and 53%), representing 22 and 24% of the total daily recovery in excreta, respectively. MEHP-derived metabolites were excreted essentially in urine (90 and 85%) and represented 26 and 23% of the daily dose or 53 and 37% of the mean total recovery within 24 hours after the 1st and 4th administration, respectively. There was a tendency to an increase of the ω -oxidation pathway between the 1st and 4th administration (38 and 51% of the total MEHP-derived metabolites excreted within 24 hours after the 1st and 4th administration, respectively) to the detriment of the ω -1-oxidation pathway (62 and 49%, respectively). Sixteen and 18% of the DEHP-metabolites were excreted in urine as glucuro-conjugates after the 1st and the 4th administration, respectively. Seven and 0.8 % of MEHP, and 19 and 25% of MEHP-derived metabolites were excreted as glucuro-conjugates within 24 hours after the 1st and the 4th administration, respectively.

Approximately 7, 32 and 31% of the daily dose were recovered in excreta as non-metabolised DEHP within 24 hours after the 1st, 4th and 7th administration of 1000 mg/kg bw/d to female CD-1 mice, representing approximately 35, 48 and 46% of the total recovery, respectively. More than 99% of non-metabolised DEHP was excreted in faeces. Within 24 hours after the 1st, 4th and 7th administration, the mean recovery of MEHP in excreta represented approximately 4, 15 and 16% of the daily dose or 20, 22 and 26% of the total recovery, respectively. Approximately 83, 92 and 92% of MEHP was excreted in urine after the 1st, 4th and 7th administration, respectively. About 9, 20 and 17% of the daily dose were recovered in excreta as MEHP-derived metabolites within 24 hours after the 1st, 4th and 7th administration. The part of the MEHP-derived metabolites in the total excretion decreased from 44% after the 1st administration to 29 and 26% after the 4th and 7th administration, respectively. Approximately 93, 97 and 93% of the MEHP-derived metabolites were excreted in urine after the 1st, 4th and 7th administration, respectively. There was a slight tendency to an increase of the ω -oxidation pathway (31, 41 and 41% of the total MEHP-derived metabolites excreted within 24 hours after the 1st, 4th and 7th administration, respectively) to the detriment of the ω -1-oxidation pathway (69, 59 and 59%, respectively) with the repetition of the administration. The proportion of MEHP-derived metabolites excreted as glucuro-conjugates in urine decreased from approximately 28% within 24 hours after the 1st administration to 10 and 13% after the 4th and 7th administration, respectively. A low part (0.4 to 4%) of the MEHP excreted in urine was glucuro-conjugated.

In conclusion, [¹⁴C]-DEHP was rapidly and extensively absorbed following a single or repeated oral administration to female mice as mirrored by the blood concentration curve profile and a rapid excretion in urine and faeces. The absorption was not dose-related. It was significantly in excess of the 5-fold difference in dose levels after a single administration and significantly below after a repeated administration. At low dose, a 5-day pre-treatment increased slightly the absorption rate, but at high dose, it induced a decrease of the absorption. After a single or a repeated administration, [¹⁴C]-DEHP was excreted very quickly in urine and faeces and the total recovery reached 60 or 69% whatever the dose level, respectively. [¹⁴C]-DEHP was excreted in majority as MEHP-derived metabolite essentially in urine, as MEHP equally in urine and faeces after a single administration and mainly in urine after a repeated administration and as DEHP almost totally in faeces. Omega-1 oxidation was the main metabolic pathway of the production of MEHP-derived metabolite. However, repeated administration of DEHP induced a metabolic activation and a displacement of the oxidation pathway in favour of the ω -oxidation. A large part of MEHP-derived metabolites were excreted as glucuro-conjugates in urine but this proportion decreased after a repeated administration of high dose. A low proportion of MEHP was excreted as glucuro-conjugate.

2. INTRODUCTION

Di-(2-ethylhexyl) phthalate (DEHP) is a phthalate ester which is also known as 1,2-benzenedicarboxylic acid di-(2-ethylhexyl) ester. It is extensively used in industry as a plasticiser for flexible polyvinyl chloride products.

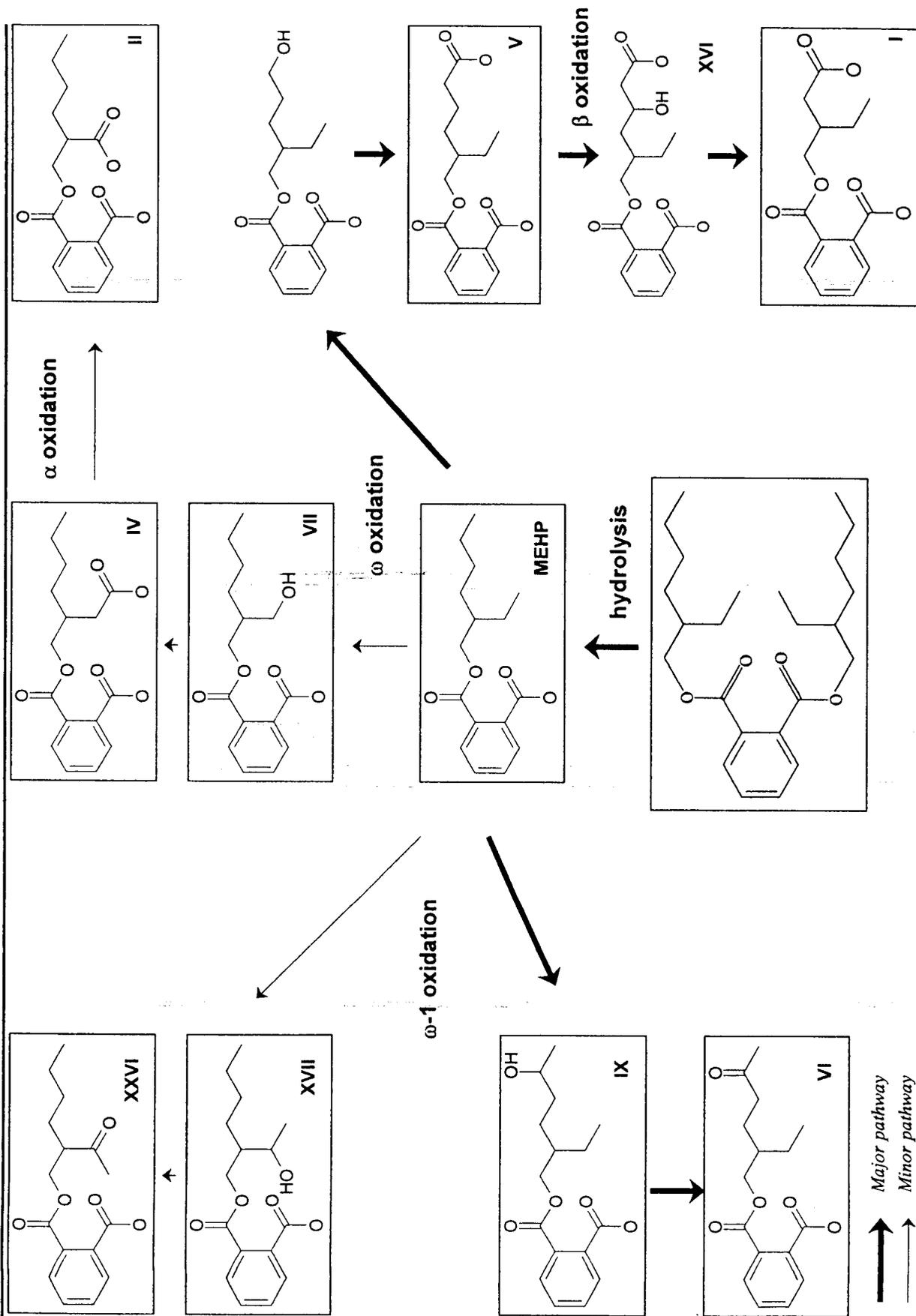


This report describes the absorption, excretion, metabolism and pharmacokinetics of DEHP in CD1 female mice after a single and repeated administration.

The structural relationships of the MEHP metabolites are summarised in the following scheme adapted from Lhuguenot et al. (1985). Only the framed metabolites have been identified in this study.

This study was performed at ENSBANA between September 1997 and April 1999.

A copy of the final report and all the primary data pertaining to the study have been retained in the Food Toxicology Laboratory of ENSBANA.



3. MATERIALS

3.1 Test Material

3.1.1 Non-radioactive test material

Unlabelled di(2-ethylhexyl)phthalate (DEHP, CAS Reg. no. 117-81-7, chemical purity > 99%) was obtained from Imperial Chemical Industries (Baleycourt, France). It was a clear, colourless liquid which arrived at ENSBANA on September 1997 and was stored at 2-8°C in the dark.

3.1.2 Radiolabelled test material

DEHP uniformly labelled on the phenyl ring ($[^{14}\text{C}]$ -DEHP, CAS Reg. no. 82208-43-3, batch 028H9474, 3.5 mCi/mmol) was purchased from Sigma (La Verpillère, France). Chemical and radiochemical purity was determined by HPLC and shown to be more than 98%.

3.2 Reagents and chemical

Mono(4-methylhexyl)phthalate (4-MHP), used as internal standard in the assays of plasma and urinary DEHP-derived metabolites, was synthesised in ENSBANA laboratory with a chemical purity greater than 95%.

Dibutyl phthalate (DBP, CAS Reg. No. 84-74-2), used as internal standard in the faecal DEHP-derived metabolites analysis, was obtained from Sigma.

Scintillation solutions, Hisafe II and Hionic Fluor, were obtained from Berthold and Wallac (France) and Packard Instrument Co. (Rungis, France), respectively. Soluene-350 was obtained from Packard Instrument Co. (Rungis, France). All the others chemicals were of the highest available purity and were purchased from Prolabo (Dijon, France).

3.3 Animals

SPF female CD1 mice were purchased from Charles River (St Aubin les Elbeuf, France). Mice were nine weeks old at the start of the experiments and their weights ranged between 25-30g.

3.4 Instrumentation

The carbon-14 was determined using an scintillation spectrometer (Packard Tri-Carb instrument, Model 2100 TR) calibrated for carbon-14 measurement using a quenched carbon-14 series and external standard spectral quench parametric analysis.

Solid sample were homogenised using an Ika-Werk homogeniser (Roucaire, Courtaboeuf, France).

DEHP-metabolites previously identified by GC-MS were quantified by GC. All chromatographic runs were performed on Chrompack CP 9000 equipped with a flame ionisation detector. Data analysis was performed by a HP Model 604 integrator. Capillary column was a Chrompack OV-1701 (stationary phase: 86% diméthyl silicon, 7% Phenyl silicon, 7% Cyanopropyl silicon).

4. METHOD

4.1 Animals

4.1.1 Identification

Each mouse was assigned a number and identified within the study by a tail-mark.

4.1.2 Acclimatisation

The mice were allowed to acclimatise to the laboratory conditions for a least three days before commencement of treatment.

4.1.3 Environmental control

The mice were housed in an environment of $23\pm 2^{\circ}\text{C}$ with a 12h dark/light cycle.

4.1.4 Animal accommodation

Animals were housed individually in metabolism cage (Nalgène) with wire mesh floors, equipped for the separate collection of urine and faeces.

Cage, cage trays, food hoppers and water bottles were changed at appropriate intervals.

4.1.5 Cage identification

Labels identifying the mouse by experiment, animal number and treatment group were placed on each cage.

4.1.6 Diet and water supply

Mice had free access to food (AO₄ pellet diet. U.A.R., Epinay sur Orge, France) and tap water.

4.2 Determination of radioactivity

The radioactivity in all samples was determined by liquid scintillation spectrometry (counting) either by direct addition of the sample to the scintillation solution (Hisafe II, Berthold and Wallac, France) or after pre-treatment.

All samples were counted until a 2σ -value equal to 1.5% is reached, or if not the maximum counting time was 5 minutes.

4.2 1 Background radioactivity

The background radioactivity was determined for each series of analyses. For liquid samples (urine and blood), the background radioactivity was determined using the addition of an equivalent volume of water to the scintillation fluid in a vial. The first vial was counted for ten minutes to determine the background radioactivity. This background was automatically subtracted from the samples counts of the considered series.

4.2.2 Limit of detection

The limit of detection (LOD) was derived statistically from the background counts so that there was 98.5% certainty that samples with a mean value greater than the limit of detection contained radioactivity from [¹⁴C]-DEHP.

The limit of detection throughout the study was approximately 3 dpm, this is equivalent to about 0.05 to 0.1 nmole DEHP-equivalents for the low dose and 0.25 to 0.5 nmole DEHP-equivalents for the high dose.

4.3 Gas chromatography analysis

Quantitative determination of the metabolite profile was performed by gas chromatography (GC).

4.3.1 Gas chromatography conditions

A 25m x 0.32mm and a 50m x 0.32mm columns were used for urine/plasma and faeces chromatographic runs, respectively. The separations were carried out with an oven temperature programmed from 150°C to 260°C at 4°C/min. Nitrogen (0.8 bar) was the carrier gas. Injector and detector port temperatures were 250°C and 280°C, respectively.

4.3.2 Quantification

Quantification was based on peak areas of the flame ionisation detector corrected as relative molar detector response when a reference compound was available, otherwise a relative molar detector response was estimated. Total amounts of parent compound and/or metabolites were calculated from radioactivity present in the urine, faeces and blood extract. In urine, blood and faeces, GC profile data was used to estimate the sum of metabolites, reaching nearly 100% of the previous amount.

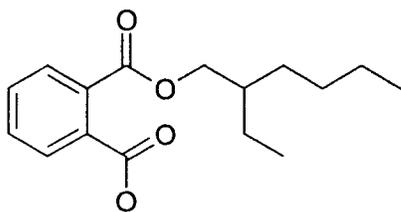
4.3.3 MEHP-derived metabolites identification

MEHP-derived metabolites were identified by gas chromatography-mass spectrometry. The detailed procedure and the results are reported in an independent report (ENSBANA addendum to reports no. 1/99 to 4/99).

The numbering proposed by Albro and Lavenhar (1989) was adopted in this study.

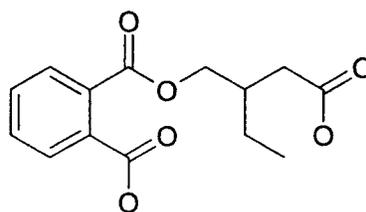
MEHP

MW: 278



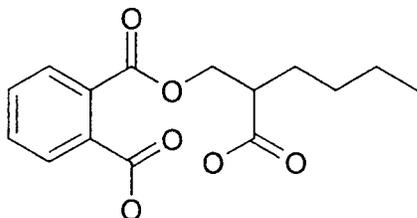
Metabolite I

MW: 280



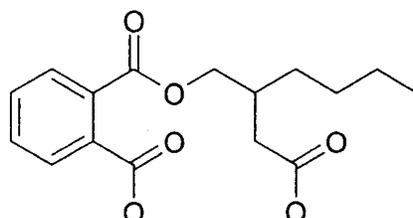
Metabolite II

MW: 294



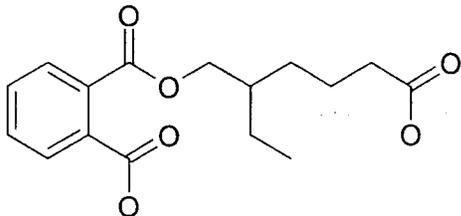
Metabolite IV

MW: 308



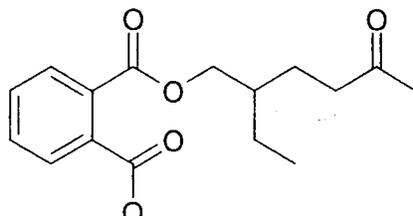
Metabolite V

MW: 308



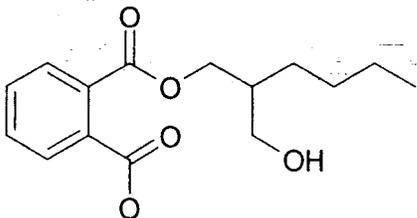
Metabolite VI

MW: 292



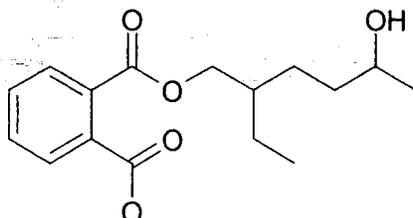
Metabolite VII

MW: 294



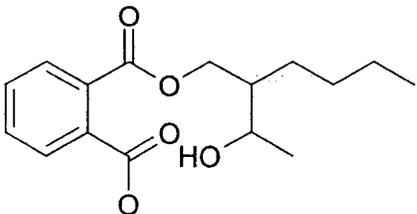
Metabolite IX

MW: 294



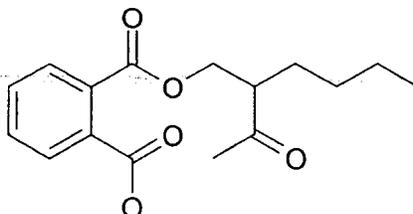
Metabolite XVII

MW: 294



Metabolite XXVI

MW: 292



MEHP :	mono-(2-ethylhexyl)phthalate
Metabolite I :	mono-(2-ethyl-3-carboxypropyl)phthalate
Metabolite II :	mono-(2-carboxyhexyl)phthalate
Metabolite IV :	mono-(2-[carboxymethyl]hexyl)phthalate
Metabolite V :	mono-(2-ethyl-5-carboxypentyl)phthalate
Metabolite VI :	mono-(2-ethyl-5-oxohexyl)phthalate
Metabolite VII :	mono-(2-[1-hydroxyethyl]hexyl)phthalate
Metabolite IX :	mono-(2-ethyl-5-hydroxyhexyl)phthalate
Metabolite XVII :	mono-(2-[2-hydroxyethyl]hexyl)phthalate
Metabolite XXVI :	mono-(2-[2-oxoethyl]hexyl)phthalate

4.4 Dose formulation

The test substance was prepared as a solution in corn oil one day before animal experimentation.

The radiochemical concentration of the stock [¹⁴C]-DEHP solution in acetone was determined by analysis in five replicate aliquots, [¹⁴C]-DEHP and unlabelled DEHP were combined at the appropriate ratio for the low and high dose to achieve the desired specific radioactivity.

The dose formulation was prepared separately for each dose by dispensing a known volume of stock liquid and adding a known volume of vehicle. The radiochemical concentration of these dose formulation was determined by analysis in five replicate aliquots.

4.5 Administration

The dose were formulated to deliver DEHP at either 200 mg/kg (15.4 µmoles/mouse) or 1000 mg/kg (76.9 µmoles/mouse) and approximately 1000000 dpm per animal in an individual dose volume of 5 ml/kg.

4.6 Treatment groups

Details of the treatment groups, types of samples and times of sampling are given below.

4.6.1 Blood radioactivity

4.6.1.1 Single oral dose

Two groups of 6 mice were selected to determine the blood radioactivity. Mice 1 to 6 received 200 mg/kg [¹⁴C]-DEHP, and mice 7 to 12 received 1000 mg/kg [¹⁴C]-DEHP with a specific radioactivity of 860,000 dpm. Immediately after administration, the mice were housed in groups of 3 in makrolon cages.

4.6.1.2 Repeated oral dose

Two groups of 15 mice were selected to investigate the effects on the blood radioactivity of a dose of [¹⁴C]-DEHP (specific radioactivity of 880,000 dpm) administered after a 5-day pre-treatment with unlabelled DEHP. Mice 13 to 24 received 200 mg/kg DEHP and mice 25 to 39 received 1000 mg/kg DEHP. Immediately after administration, the mice were housed in groups of 3 in makrolon cages.

4.6.2 Mass balance excretion study

4.6.2.1 Single oral dose

Two groups of 6 mice were allocated to the mass balance excretion study. Mice 1 to 6 received 200 mg/kg DEHP and mice 7 to 12 received 1000 mg/kg DEHP with a specific radioactivity of 1284025 dpm and 1333206 dpm, respectively.

Immediately after administration, the mice were housed by two in metabolism cages. Urine and faeces were collected at 24, 48, 72 and 96 hours after dosing.

4.6.2.2 Repeated oral dose

Two groups of 6 mice were selected to investigate the effect of multiple administration of [¹⁴C]-DEHP on the excretion study of radioactive material. Mice received single daily doses of [¹⁴C]-DEHP (584909 dpm/animal) for 9 consecutive days at 200 mg/kg/d (number 1 to 6) or 1000 mg/kg/d (number 7 to 12).

Immediately after the first administration, the mice were housed by three in metabolism cages. Urine and faeces were collected 24 hours after each dosing.

4.7 Body weights

Body weights were recorded daily during pre-test and treatment period.

4.8 Sampling and storage

4.8.1 Identification

All samples were labelled with the mouse number, time of sampling and identity of sample.

4.8.2 Urine and faeces

Urine and faeces were collected separately from the metabolism cage. The samples were kept cold during the collection periods by surrounding the collection vessels with dry ice. At the end of each study period the faecal pellets were removed from the metabolism cage. The cages were then carefully rinsed with distilled water and this was added to the urine.

For the single oral dose study, the urine of two mice was collected (group A : mice 1+2, group B : mice 3+4, group C : mice 5+6, group D : mice 7+8, group E : mice 9+10 and group F : mice 11+12). For the repeated oral dose study, the urine of three mice was collected (group A : mice 1+2+3, group B : mice 4+5+6, group C : mice 7+8+9 and group D : mice 10+11+12). Urine was diluted with distilled water to a final volume of 10ml, centrifuged, counted for ¹⁴C and then stored at -40°C until further analysis.

The sampling of faeces was the same as urine. Faeces were weighted, homogenised with distilled water (approximately a 20% homogenate) in a Potter homogeniser equipped with a teflon pestle, counted for ^{14}C and then stored at -40°C until further analysis.

4.8.3 Blood and plasma

Blood samples were taken from the orbital sinus of the animals and collected into tubes containing heparine.

After a single administration, mice numbers 1 to 3 and 7 to 9 were sampled 0.5, 1.5 and 24 hours after dosing and 4 to 6 and 10 to 12 were sampled 1, 4 and 48 hours after dosing.

After a repeated administration, mice numbers 13 to 15 were sampled 0.5 and 24 hours after dosing, 25 to 27 were sampled 0.5 and 48 hours after dosing, 16 to 18 were sampled 1 hours and 48 hours after dosing, 28 to 30 were sampled 1 hours after dosing, 19 to 21 and 31 to 33 were sampled 1.5 hours after dosing, 22 to 24 and 34 to 36 were sampled 4 hours after dosing and 37 to 39 were sampled 24 hours after dosing.

Blood samples were collected, weighed, centrifuged (250 rpm, 10 min) and plasma samples were stored at -40°C until analysis.

4.9 Sample analysis

4.9.1 Radioactivity analysis

4.9.1.1 Dose solution

Five aliquots of each solution (100 μl) were counted in mini-vials using 4 ml of scintillation solution (Hisafe II).

4.9.1.2 Urine

Aliquots of urine (100 or 200 μl) were counted in triplicate in mini-vials using 4ml of scintillation solution.

4.9.1.3 Faeces

For quantification of total radioactivity, aliquots of homogenate faeces (400 μl) were placed in triplicate in glass scintillation vials. To these were added 1ml Soluene-350. The digestion was conducted for 2h at 50°C . The digest was allowed to cool to room temperature and 0.5ml isopropanol added. Bleaching was performed by the addition of 0.2ml hydrogen peroxide (30%). The vials were incubated at 50°C for 2h. After the addition of 5ml scintillation solution (Hionic Fluor, Packard), the radioactivity was determined one day later.

For quantification of [^{14}C]-labelled DEHP, one or 2.0 ml of homogenate faeces was added to 3.0 ml of acetonitrile and 2.0 ml of hexane. The mixture was sonicated and shaken for 20 min. After centrifugation at 1000g, the hexane phase was transferred to another tube, evaporated under nitrogen and counted for ^{14}C with 4 ml of scintillation solution (Hisafe II). This method for extraction of DEHP resulted in the recovery of 93% of ^{14}C -labelled DEHP.

4.9.1.4 Blood

Total plasma collected was counted in mini-vials using 2ml of scintillation solution (Hisafe II).

4.9.2 Gas chromatography analysis

4.9.2.1 Urine

Urine was acidified to pH 2 with HCl 1M. After addition of 100 µl of internal standard solution [5.0 mg/ml 4-[MHP] in methanol], urine was extracted three fold with diethyl-ether (urine/solvent, 2.5/5 v/v). The organic extract was dried with anhydrous Na₂SO₄, evaporated to dryness in a rotary evaporator and finally dissolved in methanol. This method resulted in the recovery of about 85% of total urinary ¹⁴C.

Extracts obtained above were examined by gas chromatography before and after urine hydrolysis. Urine were diluted 1:2 with 0.1M acetate buffer pH 5.2 and incubated with 5000 U of pure bovine liver β-glucuronidase (1ml Glucurase, Sigma) overnight at 37°C. The incubation mixtures were subjected to extraction as described above.

4.9.2.2 Faeces

One or 2.0 ml of homogenate faeces was added to 3.0 ml of acetonitrile and 2.0 ml of hexane. The mixture was sonicated and shaken for 20 min. After centrifugation at 1000g, the aqueous-acetonitrile phase was acidified to pH 2 with HCl 1M and 100 µl of internal standard solution [1.0 mg/ml DBP in methanol] was added. The mixture was extracted twice with 5ml of diethyl-ether. The combined organic phases were dried and the solvent was evaporated to dryness in a rotary evaporator. Finally, the residue was dissolved in methanol. This method for extraction gave a recovery of 95% of DEHP-derived metabolites.

4.9.2.3 Preparation of derivatives

Extraction residues of urine and faeces were subjected to derivatisation. Methyl esters were prepared by treatment with a freshly prepared diazomethane/ether solution (De Boer and Becker, 1954) for at least 2h at room temperature. Excess reagent was evaporated under nitrogen and the remaining residue dissolved in 50 or 100 µl methanol before analysis by GC.

4.10 Pharmacokinetic data analysis

Pharmacokinetic data analysis were performed using the Innaphase KINETICA modelling program (Champs sur Marne, France). The non-compartmental analysis (NCA) was used to determine the toxicokinetic parameters. The area under the blood concentration-time curve (AUC) from time zero to the last data point was calculated by the trapezoidal method.

5. RESULTS

5.1 Pharmacokinetic study

5.1.1 Single dosing

5.1.1.1 Body weights

No body weight gain was observed after the treatment with 200 mg/kg and a decrease of the body weight appeared after 1000 mg/kg (table 1, appendix 1).

5.1.1.2 Recovery of radioactivity

5.1.1.2.1 Dose level 200 mg/kg

The mean concentration of radioactivity in blood (figure 1, table 2, appendix 2) decreased from a maximum of 154 ± 17 nmole DEHP-equivalents/g at 0.5 hour to 98 ± 38 nmole DEHP-equivalents/g at 1 hour. Between 1.5 hours and 4 hours the levels were stable (96 and 110 nmole DEHP-equivalents at 1.5 and 4 hours, respectively) then decreased to 25 ± 7 nmole DEHP-equivalents/g at 24 hours and finally slowly to 1 ± 0.2 nmole DEHP-equivalents/g at 48 hours.

The half-life of elimination was 7.1 hours and the area under the curve (AUC_{0-48h}) was 2069 nmole DEHP-equivalents.h.g⁻¹.

5.1.1.2.2 Dose level 1000 mg/kg

The mean concentration of radioactivity in blood (figure 1, table 2, appendix 2) decreased quickly from a maximum of 1339 ± 521 nmole DEHP-equivalents/g at 1.5 hours to 271 ± 40 nmole DEHP-equivalents/g at 4 hours and then slowly to 58 ± 29 nmole DEHP-equivalents/g at 24 hours and 14 ± 10 nmole DEHP-equivalents/g at 48 hours.

The half-life of elimination was 10.3 hours and the AUC_{0-48h} was 6838 nmole DEHP-equivalents.h.g⁻¹.

5.1.2 Repeated dosing

5.1.2.1 Body weights

No body weight gain was observed during and after the treatment period at 200 and 1000 mg/kg/d (table 3, appendixes 3 and 4).

5.1.2.2 Recovery of radioactivity

5.1.2.2.1 Dose level 200 mg/kg

Following a single oral administration of 200 mg/kg of [¹⁴C]-DEHP, after a 5-day pre-treatment with unlabelled DEHP, the mean concentration of radioactivity in blood (figure 2, table 4, appendix 5) decreased slowly from a maximum of 197 ± 60 nmole DEHP-equivalents/g at 1.5 hours to 155 ± 63 nmole DEHP-equivalents/g at 4 hours, 4 ± 1 nmole DEHP-equivalents/g at 24 hours and to 2 ± 2

nmole DEHP-equivalents at 48 hours.

The half-life of elimination was estimated to be 7.1 hours and the AUC_{0-48h} was 2252 nmole DEHP-equivalents.h.g⁻¹.

5.1.2.2.2 Dose level 1000 mg/kg

Following a single oral administration of 1000 mg/kg of [¹⁴C]-DEHP, after a 5-day pre-treatment with unlabelled DEHP, the mean concentration of radioactivity in blood (figure 2, table 4, appendix 5) decreased from a maximum of 396 ± 66 nmole DEHP-equivalents/g at 4 hours to 20 ± 17 nmole DEHP-equivalents/g at 24 hours and to 6 ± 3 at 48 hours.

The half-life of elimination was 7.9 hours and the AUC_{0-48h} was 5672 nmole DEHP-equivalents.h.g⁻¹.

5.2 Excretion and metabolism study

5.2.1 Single dosing

5.2.1.1 Body weights

No significant difference of the body weight gain of the mice was observed following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP compared to control group (table 5, appendix 6).

5.2.1.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces

5.2.1.2.1 Recovery of radioactivity

5.2.1.2.1.1 Dose level 200 mg/kg

The mean 0-96 hour recovery of radioactivity (as a percentage of radiochemical dose) was 44.4 ± 10.4% in urine and 15.4 ± 6.2% in faeces. The total mean recovery in excreta was 59.7 ± 7.0% (figure 3, table 6, appendixes 7 and 9).

During the first 24 hours, 30.9 ± 6.9 % of the radiochemical dose were excreted in urine and 10.5 ± 5.6 % in faeces (the total recovery was 41.4 ± 8.8 %). At the final collection period (72-96 hour) urine contained 1.3 ± 0.6 % and faeces 0.6 ± 0.5 % of the radiochemical dose.

5.2.1.2.1.2 Dose level 1000 mg/kg

The mean 0-96 hour recovery of radioactivity (as a percentage of radiochemical dose) was 31.0 ± 5.5% in urine and 28.2 ± 7.5% in faeces. The total mean recovery in excreta was 59.2 ± 11.1% (figure 3, table 7, appendixes 8 and 10).

During the first 24 hours, 18.4 ± 3.8% of the radiochemical dose were excreted in urine and 23.9 ± 9.2% in faeces (the total recovery was 42.3 ± 12.1%). At the final collection period (72-96 hour) urine contained 1.8 ± 0.3 % and faeces 0.3 ± 0.1% of the radiochemical dose.

5.2.1.2.2 Analysis by gas chromatography

5.2.1.2.2.1 Dose level 200 mg/kg

Urine (figures 4 to 7, table 8, appendixes 11 and 13)

MEHP-derived metabolites were the main compounds excreted in urine from female mice following a single oral administration of 200 mg/kg and accounted for 35.7% of the DEHP dose. Excretion of MEHP and DEHP represented $6.7 \pm 2.2\%$ and $0.4 \pm 0.1\%$ of the dose, respectively.

DEHP, MEHP and MEHP-derived metabolites were mainly excreted during the first 24 hours. At the final collection period (48-72 hour) urine contained $2.8 \pm 1.6\%$ of the DEHP dose as DEHP, MEHP and MEHP-derived metabolites.

Among the metabolites from MEHP ω -1-oxidation (VI, IX, XVII and XXVI), metabolites IX and VI accounted for $13.0 \pm 4.8\%$ and $8.7 \pm 2.7\%$ of the DEHP dose. Metabolite I with $5.8 \pm 1.9\%$ of the dose was the main metabolite from MEHP ω -oxidation (I, II, IV, V and VII). Metabolites II, IV, V, VII, XVII, and XXVI were excreted at low levels (0.4-2.5% of the DEHP dose) in the urine. In total, metabolites from ω -1- and ω -oxidation represented 70 and 30% of the MEHP-derived metabolites or 25 and 10% of the DEHP dose, respectively.

Fifty-four percent of the DEHP-metabolites were excreted as glucuro-conjugates (figure 6). No differences in phase II glucuronidation were observed between ω - and ω -1-oxidation pathway metabolites (figure 7).

Faeces (figures 9 and 10, table 9, appendixes 15, 17 and 19)

Expressed in % of the DEHP dose, MEHP ($6.7 \pm 2.6\%$) and DEHP ($4.0 \pm 3.0\%$) were the main compounds excreted in faeces of female mice after a single administration of 200 mg/kg. MEHP-derived metabolites (I, V, VII and VI+IX) were recovered at low levels (0.4-1.9%) (figures 9 and 10).

DEHP, MEHP and MEHP-derived metabolites were mainly excreted during the first 24 hours. At the final collection period (48-72 hour) faeces contained $1.6 \pm 0.3\%$ of the DEHP dose as DEHP, MEHP and MEHP-derived metabolites.

Total excretion of metabolites from MEHP ω -1 oxidation (VI and IX) and ω -oxidation (I, V and VII), represented 45 and 55% of the total of the MEHP-derived metabolites, but only 1.9 and 2.2% of the DEHP dose.

Total excretion (figure 11)

After a single administration of 200 mg/kg DEHP to female mice, the total excretion of DEHP and MEHP represented 4.4% and 13.4% of the DEHP dose, or 7.6 and 23% of the total compounds excreted in urine and faeces, respectively. Excretion of MEHP-derived metabolites in urine and faeces represented approximately 40% of the DEHP dose or 69.4% of the total excretion.

Metabolites from MEHP- ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII) represented 68 and 32% of the MEHP-derived metabolites excreted or 27 and 13% of the DEHP dose, respectively.

5.2.1.2.2.2 Dose level 1000 mg/kg

Urine (figures 4, 5, 6 and 8, table 10, appendixes 12 and 14)

MEHP-derived metabolites were the main compounds excreted in urine from female mice following a

single oral administration of 200 mg/kg and accounted for 22.4% of the DEHP dose. Excretion of MEHP and DEHP represented $6.5 \pm 1.9\%$ and $0.2 \pm 0.1\%$ of the dose, respectively.

DEHP, MEHP and MEHP-derived metabolites were mainly excreted during the first 24 hours. At the final collection period (48-72 hour) urine contained $2.8 \pm 0.5\%$ of the DEHP dose as DEHP, MEHP and MEHP-derived metabolites.

Among the metabolites from MEHP ω -1-oxidation (VI, IX, XVII and XXVI), metabolites IX and VI accounted for $8.9 \pm 2.6\%$ and $5.1 \pm 1.8\%$ of the DEHP dose. Metabolite I with $3.7 \pm 1.4\%$ of the dose was the main metabolite from MEHP ω -oxidation (I, II, IV, V and VII). Metabolites II, IV, V, VII, XVII, and XXVI were excreted at low levels (0.3-1.5% of the DEHP dose) in the urine. In total, metabolites from ω -1- and ω -oxidation represented 71 and 29% of the MEHP-derived metabolites or 16 and 6.5% of the DEHP dose, respectively.

Twenty-one percent of the DEHP-metabolites were excreted as glucuro-conjugates (figure 6). Forty-nine, 15 and 9% of MEHP, ω -1-oxidation and ω -oxidation metabolites were excreted as glucuro-conjugated, respectively (figure 8).

Faeces (figures 9 and 10, table 11, appendixes 16, 18 and 20)

Expressed in % of the DEHP dose, MEHP ($5.8 \pm 3.7\%$) and DEHP ($18.1 \pm 9.3\%$) were the main compounds excreted in faeces of female mice after a single administration of 1000 mg/kg. MEHP-derived metabolites (I, V, VII and VI+IX) were recovered at low levels (0.1-2.2%) (figures 9 and 10).

DEHP, MEHP and MEHP-derived metabolites were mainly excreted during the first 24 hours. At the final collection period (48-72 hour) faeces contained $0.7 \pm 0.3\%$ of the DEHP dose as DEHP, MEHP and MEHP-derived metabolites.

Metabolites from MEHP ω -1 oxidation (VI and IX) and ω -oxidation (I, V and VII), represented 55 and 45% of the total of the MEHP-derived metabolites excreted in faeces, but only 2.2 and 1.8% of the DEHP dose.

Total excretion (figure 11)

After a single administration of 1000 mg/kg DEHP to female mice, the total excretion in urine and faeces of DEHP and MEHP represented 18.3% and 12.3% of the administered DEHP dose, or 32.1 and 21.6% of the compounds excreted, respectively. Approximately 26% of the DEHP dose were excreted as MEHP-derived metabolites.

Metabolites from MEHP- ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII) represented 69 and 31% of the MEHP-derived metabolites or 18 and 8% of the DEHP dose, respectively.

5.2.2 Repeated dosing

5.2.2.1 Body weights

Compared to the control mice, no significant effect on the body weight gain was observed at 200 mg/kg/d. At 1000 mg/kg/d, the body weight gain of the mice was slightly reduced from the 2nd administration and afterwards, mouse #12 died after the 4th administration (table 12, appendix 21).

5.2.2.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces

5.2.2.2.1 Recovery of radioactivity

5.2.2.2.1.1 Dose level 200 mg/kg

Urine (figures 12 and 13, table 13, appendixes 22 and 23)

The mean recovery of radioactivity in urine was 5.9 ± 0.9 $\mu\text{mole DEHP-equivalents}$ per day during the treatment period (from 4.3 ± 0.9 $\mu\text{mole-DEHP equivalents}$ within 24 hours after the 4th administration up to 6.9 ± 2.0 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 2nd administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly and only 1.2 ± 0.6 $\mu\text{mole DEHP-equivalents}$ were excreted during the last urine collection period (24-48 hours after the 9th administration).

The total recovery of radioactivity in urine was $36.2 \pm 5.2\%$ of the total administered dose.

Faeces (figures 12 and 13, table 14, appendixes 22 and 23)

The mean recovery of radioactivity in faeces was 4.8 ± 1.2 $\mu\text{mole DEHP-equivalents}$ per day during the treatment period (from 2.6 ± 1.9 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 1st administration up to 6.9 ± 1.3 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 5th administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly, and only 0.3 ± 0.3 $\mu\text{mole DEHP-equivalents}$ were excreted during the last collection period (24-48 hours after the 9th administration).

The total recovery of radioactivity in faeces was $32.0 \pm 7.0\%$ of the total administered dose.

Total excretion (figures 12 and 13, table 15)

The mean recovery of radioactivity in excreta was 10.0 ± 2.6 $\mu\text{mole DEHP-equivalents}$ per day during the treatment period (from 4.8 ± 1.5 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 7th administration up to 12.6 ± 2.6 $\mu\text{mole-DEHP equivalents}$ within 24 hours after the 5th administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly and only 1.5 ± 0.3 $\mu\text{mole DEHP-equivalents}$ were excreted during the last excreta collection period (24-48 hours after the 9th administration).

The total recovery of radioactivity in excreta was $68.1 \pm 1.8\%$ of the total administered dose.

5.2.2.2.1.2 Dose level 1000 mg/kg

Urine (figures 14 and 15, table 16, appendixes 24 and 25)

The mean recovery of radioactivity in urine was 26.5 ± 7.5 $\mu\text{mole DEHP-equivalents}$ per day during the treatment period (from 9.2 ± 2.2 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 1st administration up to 37.3 ± 10.4 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 2nd administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly and only 4.9 ± 2.3 $\mu\text{mole DEHP-equivalents}$ were excreted during the last urine collection period (24-48 hours after the 9th administration).

The total recovery of radioactivity in urine was $36.7 \pm 1.4\%$ of the total administered dose.

Faeces (figures 14 and 15, table 17, appendixes 24 and 25)

The mean recovery of radioactivity in faeces was 24.9 ± 8.8 μ mole DEHP-equivalents per day during the treatment period (from 6.3 ± 3.6 μ mole DEHP-equivalents within 24 hours after the 1st administration up to 34.9 ± 6.6 μ mole DEHP-equivalents within 24 hours after the 2nd administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly and only 0.8 ± 0.3 μ mole-DEHP equivalents were excreted during the last faeces collection period (24-48 hours after the 9th administration).

The total recovery of radioactivity in faeces was $32.7 \pm 2.2\%$ of the total administered dose.

Total excretion (figures 14 and 15, table 18)

The mean recovery of radioactivity in excreta was 51.4 ± 15.9 μ mole DEHP-equivalents per day during the treatment period (from 15.5 ± 5.8 μ mole DEHP-equivalents within 24 hours after the 1st administration up to 72.2 ± 3.7 μ mole DEHP-equivalents within 24 hours after the 2nd administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly and only 5.7 ± 2.0 μ mole-DEHP equivalents were excreted during the last excreta collection period (24-48 hours after the 9th administration).

The total recovery of radioactivity in excreta was $69.4 \pm 0.9\%$ of the total administered dose.

5.2.2.2 Analysis by gas chromatography

5.2.2.2.1 Dose level 200 mg/kg

Urine (Figure 16, 17 and 18, table 19, appendixes 26 and 28)

The urine samples collected after the 7th administration were lost. Only the urine samples collected after the 1st and 4th administration have been analysed.

Only trace amounts of DEHP were detected in urine, 0.05 ± 0.04 and 0.07 ± 0.04 μ moles within 24 hours after the 1st and 4th administration, respectively.

Within 24 hours after the 1st administration, MEHP was excreted at levels of 1.19 ± 0.19 μ moles in urine. After the 4th administration, the corresponding values were 1.21 ± 0.31 μ moles.

DEHP and MEHP represented 1.0 and 1.6%, and 24.8 and 28.1% of the compounds excreted after the 1st and 4th administration, respectively.

MEHP-derived metabolites were excreted at levels of 3.56 and 3.02 μ moles after the 1st and the 4th administration, these levels represented 74 and 70% of the total excretion, respectively.

Metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) were excreted in urine at levels of 2.35 and 1.62 μ moles within 24 hours after the 1st and 4th administration. These metabolites represented 66 and 60% of the MEHP-derived metabolites or 49 and 38% of the total excretion within 24 hours after the 1st and the 4th administration, respectively.

Metabolites from MEHP ω -oxidation (I, II, IV, V and VII) were excreted in urine at levels of 1.21 and 1.4 μ moles within 24 hours after the 1st and 4th administration. These metabolites represented 34 and 40% of the MEHP-derived metabolites or 25 and 32% of the total excretion after the 1st and 4th administration, respectively.

Sixteen and 18% of the DEHP-metabolites were excreted as glucuro-conjugates after the 1st and the 4th administration (figure 17), respectively. Seven and 0.8 % of MEHP, 16 and 31% of ω -1-oxidation metabolites and 23 and 15% of ω -oxidation metabolites were excreted as glucuro-conjugates after the 1st and the 4th administration, respectively (figure 18).

Faeces (figure 19, table 20, appendixes 30, 31 and 33)

DEHP and MEHP were the main compounds recovered in faeces from female mice following a repeated oral administration of 200 mg/kg bw/d DEHP.

The faecal excretion of DEHP increased with time from 1.93 ± 1.22 μ moles after the 1st administration to 3.43 ± 1.98 and 4.05 ± 1.64 μ moles after the 4th and 7th administration, respectively. DEHP represented 73, 66 and 84% of the total faecal excretion after the 1st, 4th and 7th administration, respectively.

The faecal excretion of MEHP was 0.48 ± 0.49 , 1.07 ± 0.06 and 0.49 ± 0.10 μ moles after the 1st, 4th and 7th administration; equivalent to 18, 20 and 10% of the total faecal excretion, respectively.

The recovery of the MEHP-derived metabolites represented 9, 13 and 5 % of the total faecal excretion after the 1st, 4th and 7th administration, respectively.

Metabolites from ω -1 oxidation (VI and IX) represented 46, 40 and 35% of the MEHP-derived metabolites or 4, 5 and 2% the total faecal excretion within 24 hours after the 1st, 4th and 7th administration, respectively.

Metabolites from ω -oxidation (I, IV, V and VII) represented 54, 60 and 65 % of MEHP-derived metabolites or 5, 8 and 3% of the total faecal excretion within 24 hours after the 1st, 4th and 7th administration, respectively.

Total excretion (figure 20)

Only the results for the 1st and 4th administration are presented due to the absence of urine samples after the 7th administration.

The excretion of non-metabolised DEHP was 1.98 and 3.5 μ moles after the 1st and 4th administration, which represented 26 and 37% of the total excretion, respectively. Ninety-seven and 98% of DEHP were excreted in faeces, respectively.

The total excretion of MEHP was 1.67 and 2.28 μ moles after the 1st and 4th administration, which accounted for 22 and 24% of the total excretion, respectively. Seventy-one and 53% of MEHP were excreted in urine, respectively.

The total excretion of MEHP-derived metabolites was 3.95 and 3.57 μ moles after the 1st and 4th administration, respectively. These metabolites represented 53 and 37% of the total excretion after the 1st and 4th administration, respectively. Ninety and 85% of these metabolites were excreted in urine within 24 hours after the 1st and 4th administration, respectively.

The total excretion of MEHP ω -1 oxidation metabolites (VI, IX, XVII and XXVI) was 2.46 and 1.75 μ moles after the 1st and 4th administration, respectively. These metabolites represented 62 and 49% of the MEHP-derived metabolites excreted within 24 hours after the 1st and 4th administration, respectively. Ninety-five and 93% of these metabolites were excreted in urine, respectively.

The total excretion of MEHP ω -oxidation metabolites (I, II, IV, V and VII) was 1.49 and 1.82 μ moles after the 1st and 4th administration, respectively. These metabolites represented 38 and 51% of the MEHP-derived metabolites excreted within 24 hours after the 1st and 4th administration, respectively. Eighty-one and 77% of these metabolites were excreted in urine, respectively.

5.2.2.2.2 Dose level 1000 mg/kg

Urine (Figures 21, 22 and 23, table 21, appendixes 27 and 29)

Only trace amounts of DEHP were detected in urine (0.04 ± 0.01 , 0.11 ± 0.05 and 0.13 ± 0.01 μ moles within 24 hours after the 1st, 4th and 7th administration, respectively). These quantities represented only 0.4, 0.4 and 0.5% of the total compounds excreted, respectively.

Within 24 hours after the 1st, 4th and 7th administration, MEHP was excreted at levels of 2.63 ± 0.35 , 10.74 ± 5.78 and 11.69 ± 2.53 μ moles in urine, respectively. These quantities represented 28, 42 and 49% of the total compounds excreted, respectively.

MEHP-derived metabolites were excreted at levels of 6.53, 14.65 and 11.83 μ moles within 24 hours after the 1st, 4th and the 7th administration. These levels represented 71, 57 and 50% of the total excretion, respectively.

Metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) were excreted in urine at levels of 4.62, 8.70 and 7.33 μ moles within 24 hours after the 1st, 4th and the 7th administration, respectively. These metabolites represented 71, 59 and 62% of MEHP-derived metabolites excreted or 50, 34 and 31% of the total excretion after the 1st, 4th and 7th administration, respectively.

Metabolites from MEHP ω -oxidation (I, II, IV, V and VII) were excreted in urine at levels of 1.91, 5.95 and 4.50 μ moles within 24 hours after the 1st, 4th and the 7th administration. These metabolites represented 29, 41 and 38% of the MEHP-derived metabolites excreted or 21, 23 and 19% of the total excretion after the 1st, 4th and 7th administration, respectively.

Twenty, 7 and 9% of the DEHP-metabolites were excreted as glucuro-conjugates after the 1st, 4th and 7th administration (figure 22), respectively. 0.4, 3 and 4% of MEHP; 31, 15 and 17% of ω -1-oxidation metabolites and 22, 2 and 10% of ω -oxidation metabolites were excreted as glucuro-conjugates after the 1st, 4th and 7th administration, respectively (figure 23).

Faeces (figure 24, table 22, appendixes 30, 32 and 34)

DEHP was the main compound excreted in faeces from female mice following a repeated oral administration of 1000 mg/kg/d DEHP. The total faecal excretion of DEHP increased significantly with the repetition of the administration, from 5.43 ± 2.84 μ moles after the 1st administration to 24.69 ± 9.55 and 23.65 ± 2.25 μ moles after the 4th and 7th administration, respectively. DEHP represented 87, 94 and 94% of the total faecal excretion after the 1st, 4th and 7th administration, respectively.

The faecal excretion of MEHP was low with mean values of 0.53 ± 0.53 , 0.95 ± 0.32 and 0.97 ± 0.15 μ moles within 24 hours after the 1st, 4th and 7th administration, equivalent to 8, 4 and 4% of the total faecal excretion, respectively.

The recovery of the MEHP-derived metabolites was low, with mean values of 0.29, 0.51 and 0.88 μ moles within 24 hours after the 1st, 4th and 7th administration and represented 5, 2 and 3 % of the excreted compounds, respectively.

Metabolites from ω -1 oxidation (VI and IX) represented 24, 47 and 16% of the total faecal excretion of MEHP-derived metabolites, or 1, 1 and 0.5% the total faecal excretion within 24 hours after the 1st, 4th and 7th administration, respectively.

Metabolites from ω -oxidation (I, IV, V and VII) represented 76, 53 and 84 % of the total faecal excretion of MEHP-derived metabolites, or 3, 1 and 3% of the total faecal excretion within 24 hours after the 1st, 4th and 7th administration, respectively.

Total excretion (figure 25)

The excretion of non-metabolised DEHP was 5.47, 24.80 and 23.78 μ moles after the 1st, 4th and 7th administration, which represented 35, 48 and 49% of the total excretion, respectively. In each case, 99% of DEHP was excreted in faeces.

The total excretion of MEHP was 3.16, 11.69 and 12.66 μ moles after the 1st, 4th and 7th administration, which accounted for 20, 22 and 26% of the total excretion, respectively. Eighty-three, 92 and 92% of MEHP were excreted in urine, respectively.

The total excretion of MEHP-derived metabolites was 6.82, 15.16 and 12.71 μ moles after the 1st, 4th and 7th administration, respectively. These metabolites represented 44, 29 and 26% of the total excretion after the 1st, 4th and 7th administration, respectively. Ninety-three, 97 and 93% of these metabolites were excreted in urine within 24 hours after the 1st, 4th and 7th administration, respectively.

The total excretion of MEHP ω -1 oxidation metabolites (VI, IX, XVII and XXVI) was 4.69, 8.94 and 7.47 μ moles after the 1st, 4th and 7th administration, respectively. These represented 69, 59 and 59% of the MEHP-derived metabolites excreted within 24 hours after the 1st, 4th and 7th administration, respectively. In each case, approximately 98% these metabolites were excreted in urine.

The total excretion of MEHP ω -oxidation metabolites (I, II, IV, V and VII) was 2.13, 6.22 and 5.24 μ moles after the 1st, 4th and 7th administration, respectively. These represented 31, 41 and 41% of the MEHP-derived metabolites excreted within 24 hours after the 1st, 4th and 7th administration, respectively. Eighty-nine, 96 and 86% of these metabolites were excreted in urine, respectively.

6. DISCUSSION

The absorption of radioactive material following single oral doses of [¹⁴C]-DEHP at 200 mg/kg and 1000 mg/kg to female CD-1 mice was rapid. The maximum blood concentrations (C_{max}), approximately 154 and 1339 nmole DEHP-equivalents/g for the low and the high doses, were obtained 0.5 hour and 1.5 hours after dosing, respectively. The half-life of elimination were approximately 7.1 and 10.3 hours at the low and the high dose level, respectively. The areas under the curves (AUC_{0-48h}) were 2069 and 6838 nmole DEHP-equivalents.h.g⁻¹ for 200 mg/kg and 1000 mg/kg dose levels, respectively. The 8.7-fold difference in the C_{max} between dose levels and the 3.3-fold difference in AUC are at variance with the 5-fold difference in dose levels. Negligible or low levels of radioactivity (approximately 1% of the C_{max}) were detected in blood samples 48 hours for the low and the high doses, respectively.

The absorption of the radioactive material was rapid, following a single dose of 200 or 1000 mg/kg [¹⁴C]-DEHP administered after a 5-day pre-treatment of female CD-1 mice with 200 or 1000 mg/kg bw/d of unlabelled DEHP. The C_{max} , approximately 197 and 396 nmole-DEHP equivalents/g for the low and the high doses, were obtained 1.5 hours and 4 hours after dosing, respectively. The half-life of elimination were approximately 7.1 and 7.9 hours at the low and the high dose level, respectively. The AUC_{0-48h} were 2252 and 5672 nmole DEHP-equivalents.h.g⁻¹ for 200 and 1000 mg/kg dose levels, respectively. This 2-fold difference in the C_{max} between dose levels and the 2.5-fold difference in AUC is at variance the 5-fold difference in dose levels and can indicate a saturation of the absorption process. Negligible or low level of radioactivity (approximately 1-1.5% of the C_{max}) were detected in blood samples at 48 hours for the low and the high doses, respectively.

According to the previous results, a 5-day pre-treatment with unlabelled DEHP at 200 mg/kg bw/d induced a 28% and 9% increase of the C_{max} and the AUC of the radioactive dose, respectively. In contrast, at 1000 mg/kg bw/d, a 5-day pre-treatment induced a 3.4-fold decrease of the C_{max} and 17% decrease of the AUC.

After a single oral administration to female CD-1 mice, excretion of radioactive material was very rapid as most was excreted in the first 48 hours. Following the low and high dose administrations, 44.4% and 31.0% of the dose were recovered in the urine and 15.4% and 28.2% in the faeces within 96 hours, respectively. The total overall mean recovery was approximately 60% at both dose levels.

Approximately 4.4 and 18.3% of the low and high DEHP dose were excreted unchanged almost totally in faeces, either due to a non absorption or/and a re-excretion as a consequence of an entero-hepatic re-circulation. Approximately 13.4 and 12.3% of the low and high doses were excreted as MEHP, the proximate metabolite issued from DEHP hydrolysis, respectively. MEHP was excreted approximately 50/50 in urine and faeces at both dose levels. Total excretion of MEHP-derived metabolites reached 40 and 26% of the dose at 200 mg/kg and 1000 mg/kg, respectively. They were essentially excreted in urine (35% and 22% of the low and high DEHP dose, respectively) and for a small part in faeces (4.1% and 4% of the low and high DEHP dose, respectively). At 200 and 1000 mg/kg, approximately 58 and 49% of MEHP, and 54 and 13% of MEHP-derived metabolites were excreted in urine as glucuro-conjugates, respectively. Whatever the dose level, MEHP ω -1 oxidation was the main metabolic pathway (about 70%), followed by ω -oxidation (about 30%).

During a 9-day repeated administration at 200 and 1000 mg/kg bw/d [¹⁴C]-DEHP to female mice, approximately 36.2 and 36.7.8% of the total [¹⁴C]-DEHP doses were recovered in the urine and 32.0 and 32.7% in the faeces, respectively. At both dose levels, daily excretion was stable with mean values of 5.9 ± 0.9 and 26.5 ± 7.5 μ mole DEHP-equivalents/day in urine and 4.8 ± 1.2 and 24.9 ± 8.8 μ mole DEHP-equivalents/day in faeces, respectively. The total overall mean recovery of radioactive material in excreta was approximately 68% at 200 mg/kg bw/d and 69% at 1000 mg/kg bw/d. These values of total excretion and the observed steady-state excluded any significant bio-accumulation of DEHP.

During the repeated administration of 200 mg/kg bw/d DEHP for 9 days to female CD-1 mice, about 13 and 23% of the DEHP daily dose were excreted almost totally in faeces (97-98%) as non-metabolised DEHP within 24 hours after the 1st and 4th administration, representing 26 and 37% of the total daily recovery in excreta, respectively. Only trace amounts of non-metabolised DEHP were detected in urine. Within 24 hours after the 1st and 4th administration, about 11 and 15% of the DEHP daily dose were excreted as MEHP mainly in urine (71 and 53%), representing 22 and 24% of the total

daily recovery in excreta, respectively. MEHP-derived metabolites were excreted essentially in urine (90 and 85%) and represented 26 and 23% of the daily dose or 53 and 37% of the mean total recovery within 24 hours after the 1st and 4th administration, respectively. There was a tendency to an increase of the ω -oxidation pathway between the 1st and 4th administration (38 and 51% of the total MEHP-derived metabolites excreted within 24 hours after the 1st and 4th administration, respectively) to the detriment of the ω -1-oxidation pathway (62 and 49%, respectively). Sixteen and 18% of the DEHP-metabolites were excreted in urine as glucuro-conjugates after the 1st and the 4th administration, respectively. Seven and 0.8 % of MEHP, and 19 and 25% of MEHP-derived metabolites were excreted as glucuro-conjugates within 24 hours after the 1st and the 4th administration, respectively.

Approximately 7, 32 and 31% of the daily dose were recovered in excreta as non-metabolised DEHP within 24 hours after the 1st, 4th and 7th administration of 1000 mg/kg bw/d to female CD-1 mice, representing approximately 35, 48 and 46% of the total recovery, respectively. More than 99% of non-metabolised DEHP was excreted in faeces. Within 24 hours after the 1st, 4th and 7th administration, the mean recovery of MEHP in excreta represented approximately 4, 15 and 16% of the daily dose or 20, 22 and 26% of the total recovery, respectively. Approximately 83, 92 and 92% of MEHP was excreted in urine after the 1st, 4th and 7th administration, respectively. About 9, 20 and 17% of the daily dose were recovered in excreta as MEHP-derived metabolites within 24 hours after the 1st, 4th and 7th administration. The part of the MEHP-derived metabolites in the total excretion decreased from 44% after the 1st administration to 29 and 26% after the 4th and 7th administration, respectively. Approximately 93, 97 and 93% of the MEHP-derived metabolites were excreted in urine after the 1st, 4th and 7th administration, respectively. There was a slight tendency to an increase of the ω -oxidation pathway (31, 41 and 41% of the total MEHP-derived metabolites excreted within 24 hours after the 1st, 4th and 7th administration, respectively) to the detriment of the ω -1-oxidation pathway (69, 59 and 59%, respectively) with the repetition of the administration. The proportion of MEHP-derived metabolites excreted as glucuro-conjugates in urine decreased from approximately 28% within 24 hours after the 1st administration to 10 and 13% after the 4th and 7th administration, respectively. A low part (0.4 to 4%) of the MEHP excreted in urine was glucuro-conjugated.

In conclusion, [¹⁴C]-DEHP was rapidly and extensively absorbed following a single or repeated oral administration to female mice as mirrored by the blood concentration curve profile and a rapid excretion in urine and faeces. The absorption was not dose-related. It was significantly in excess of the 5-fold difference in dose levels after a single administration and significantly below after a repeated administration. At low dose, a 5-day pre-treatment increased slightly the absorption rate, but at high dose, it induced a decrease of the absorption. After a single or a repeated administration, [¹⁴C]-DEHP was excreted very quickly in urine and faeces and the total recovery reached 60 or 69% whatever the dose level, respectively. [¹⁴C]-DEHP was excreted in majority as MEHP-derived metabolite essentially in urine, as MEHP equally in urine and faeces after a single administration and mainly in urine after a repeated administration and as DEHP almost totally in faeces. Omega-1 oxidation was the main metabolic pathway of the production of MEHP-derived metabolite. However, repeated administration of DEHP induced a metabolic activation and a displacement of the oxidation pathway in favour of the ω -oxidation. A large part of MEHP-derived metabolites were excreted as glucuro-conjugates in urine but this proportion decreased after a repeated administration of high dose. A low proportion of MEHP was excreted as glucuro-conjugate.

7. REFERENCES

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Lhuguenot. J. C.; Mitchell. A. M.; Milner. G.; Lock. E. A.; Elcombe. C. R. (1985) The metabolism of di(2-ethylhexyl) phthalate (DEHP) and mono-(2-ethylhexyl) phthalate (MEHP) in mice: *in vivo* and *in vitro* dose and time dependency of metabolism. Toxicol. Appl. Pharmacol., 80(1). 11-22.

FIGURES

Pharmacokinetic study

Figure 1. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice following a single oral administration of [¹⁴C]-DEHP at 200 or 1000 mg/kg.

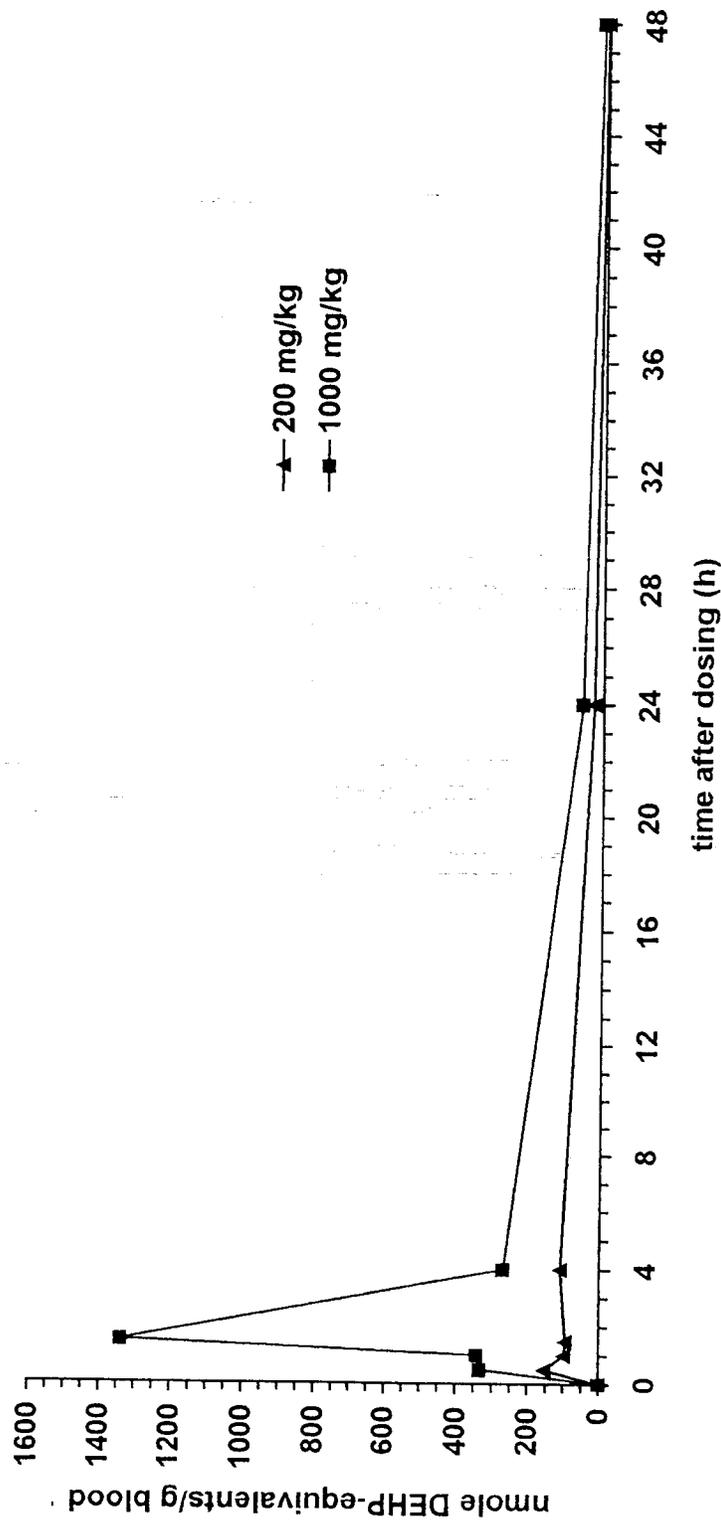
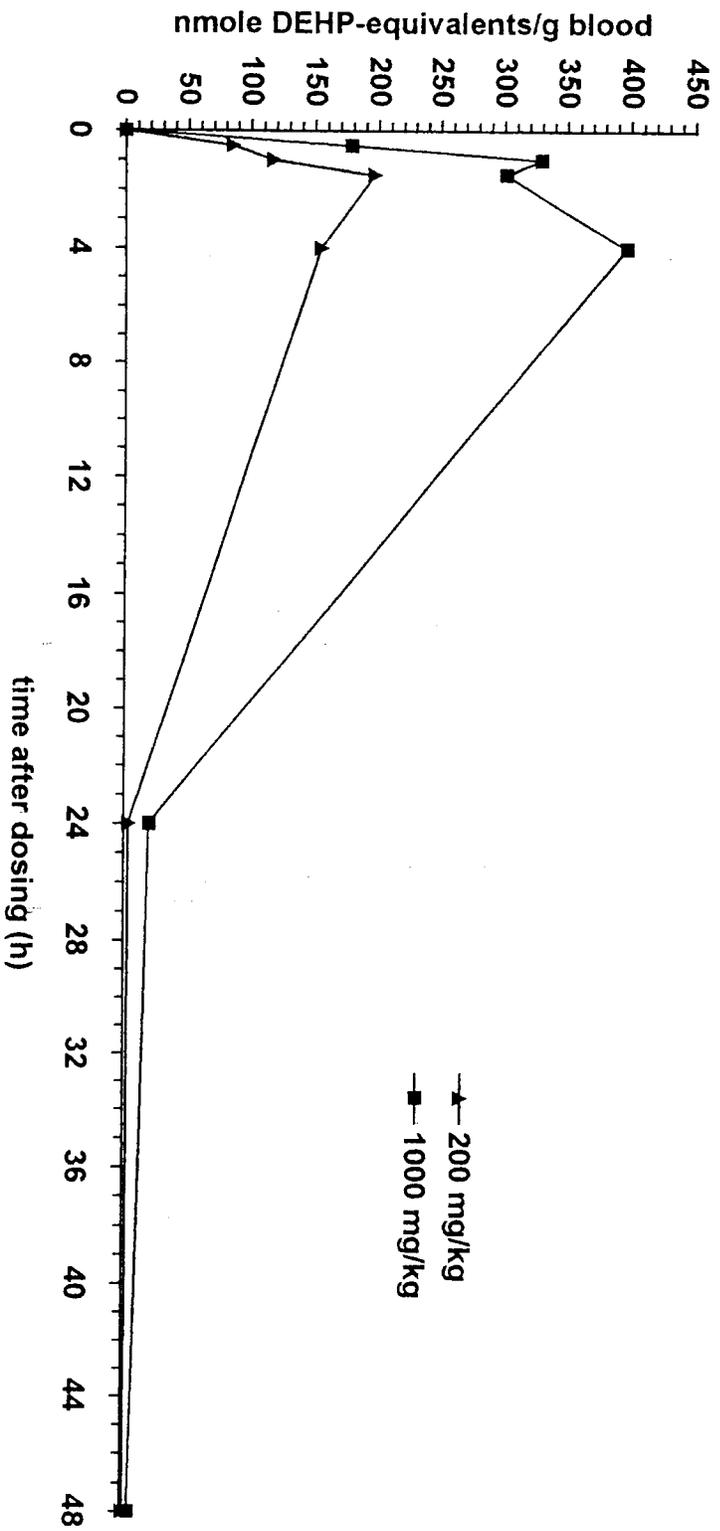


Figure 2. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice following a single oral administration of 200 or 1000 mg/kg [¹⁴C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled-DEHP, respectively.



Excretion and metabolism study, single dosing.

Figure 3. Recovery of radioactivity (in % of the DEHP dose) in urine and faeces from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg [¹⁴C]-DEHP.

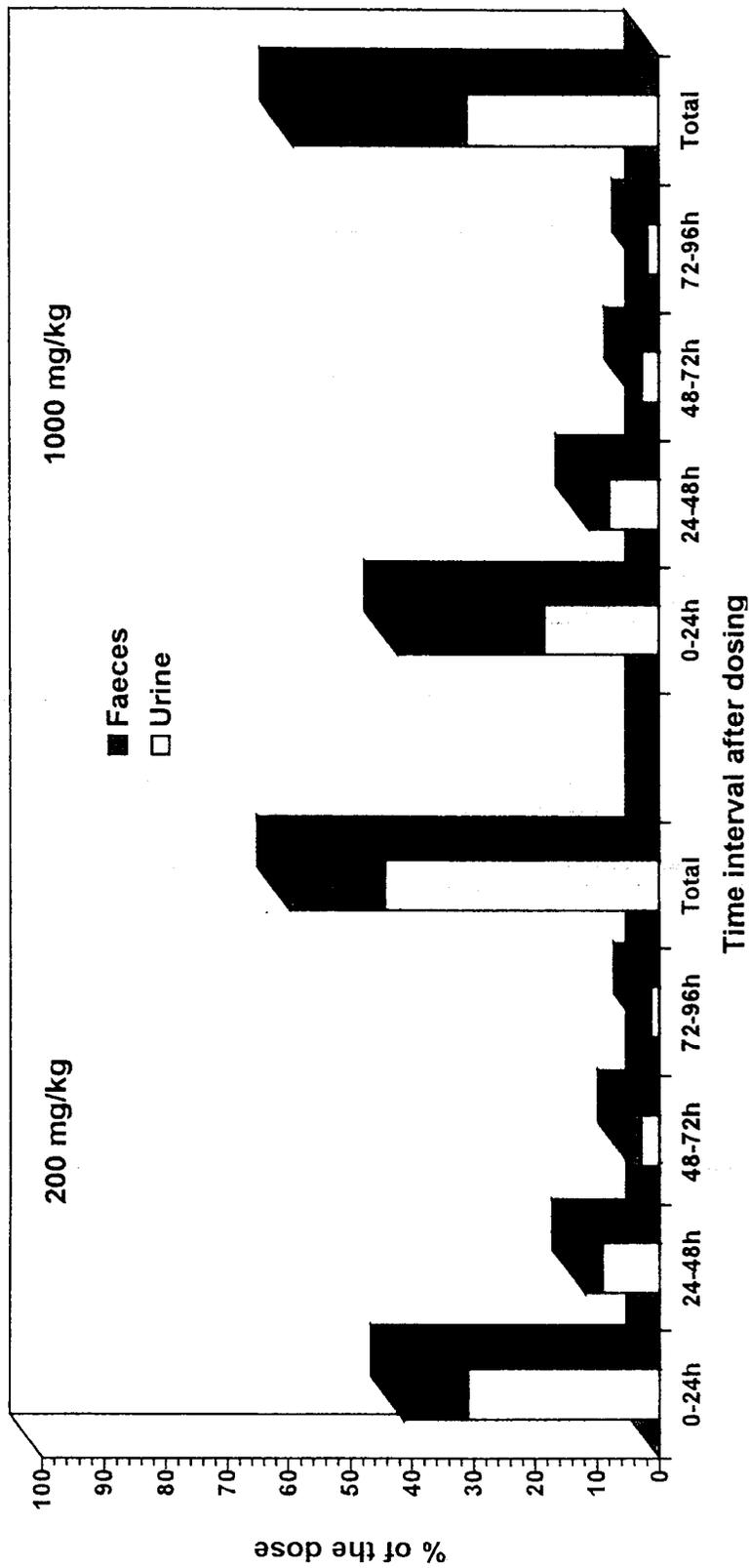


Figure 4. Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

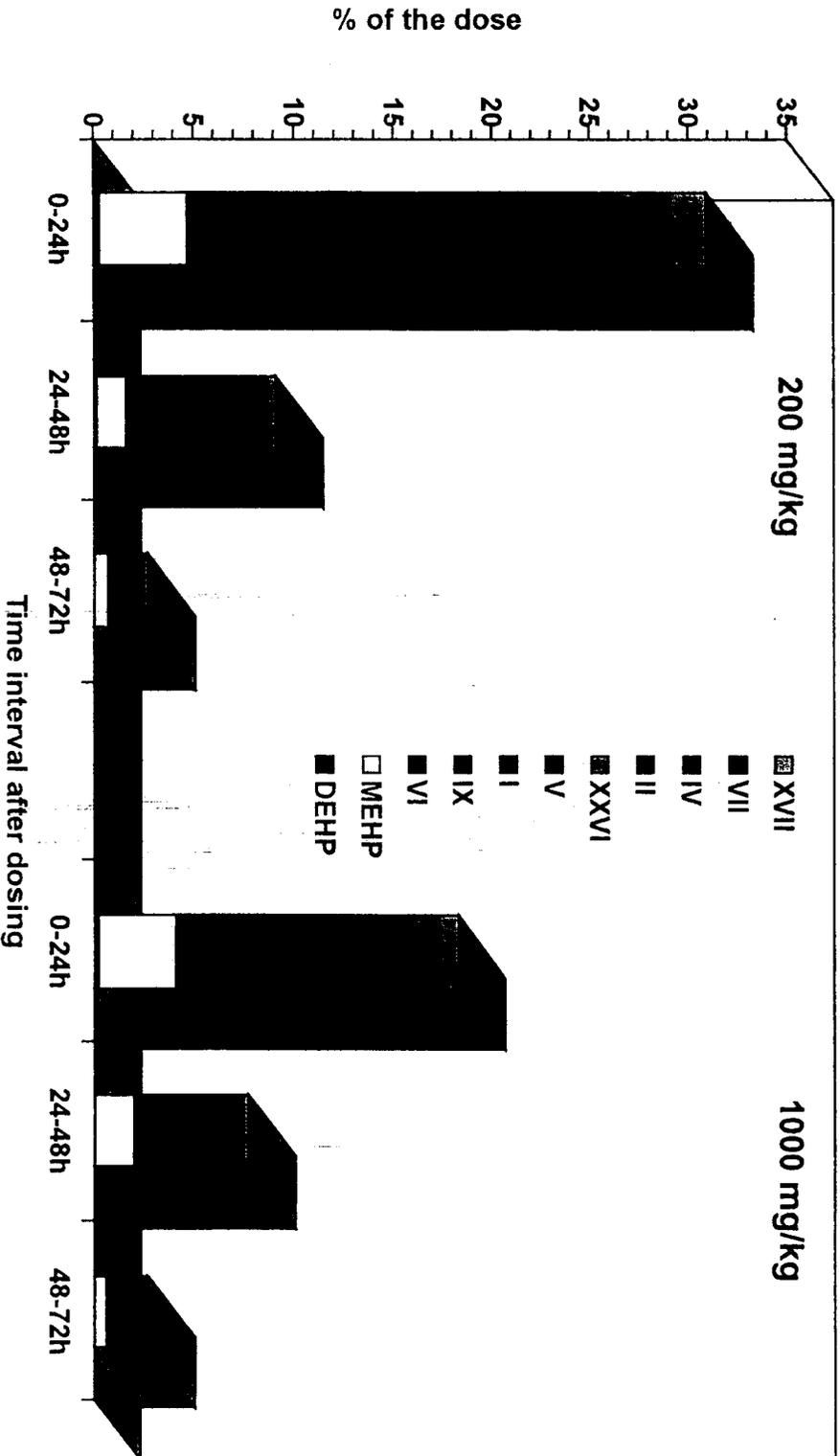


Figure 5. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

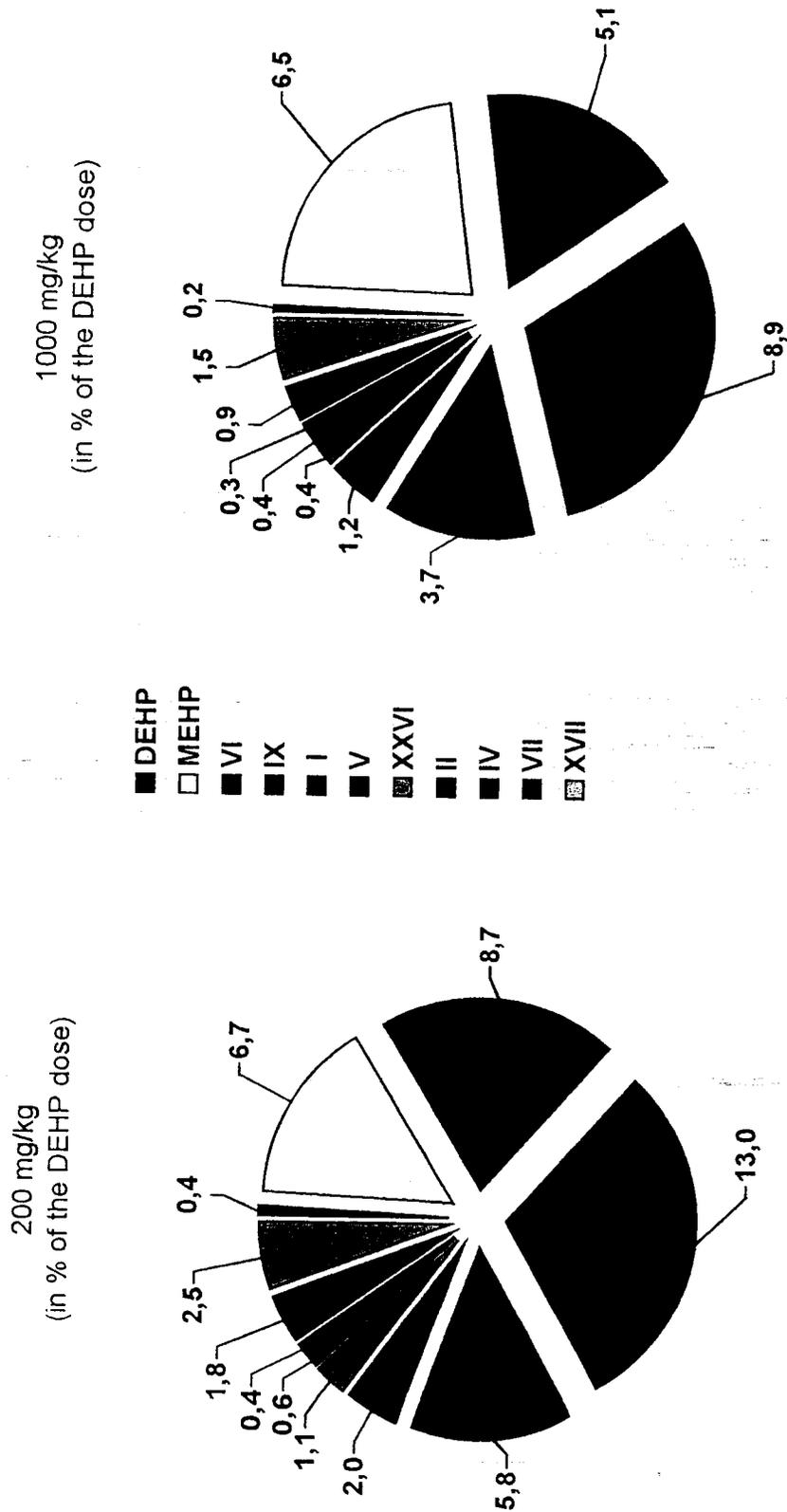


Figure 6. Distribution of free and conjugate metabolites in urine from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg

Figure 6. Distribution of free and conjugate metabolites in urine from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

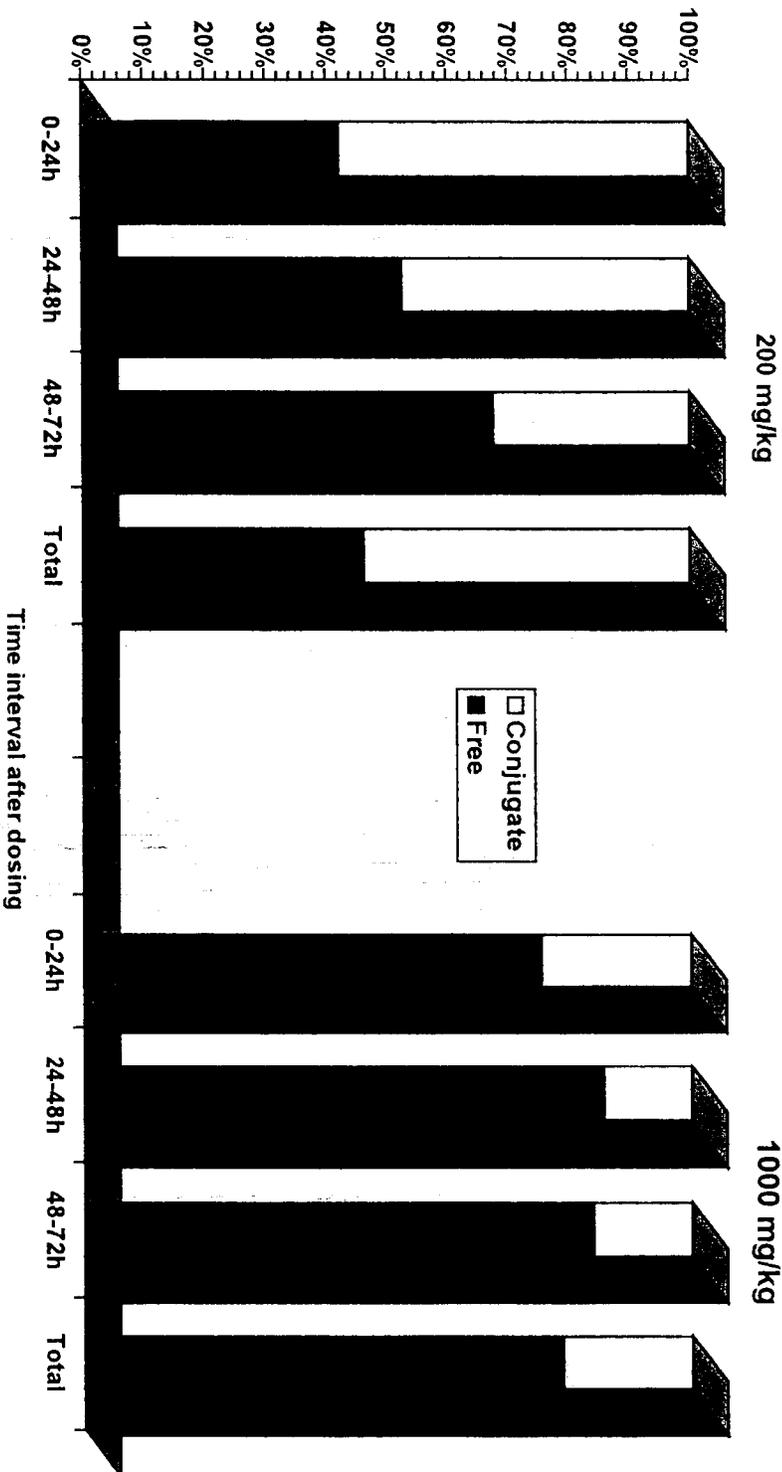


Figure 7. Cumulative excretion (0-72h) and distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female mice following a single oral administration of 200 mg/kg DEHP.

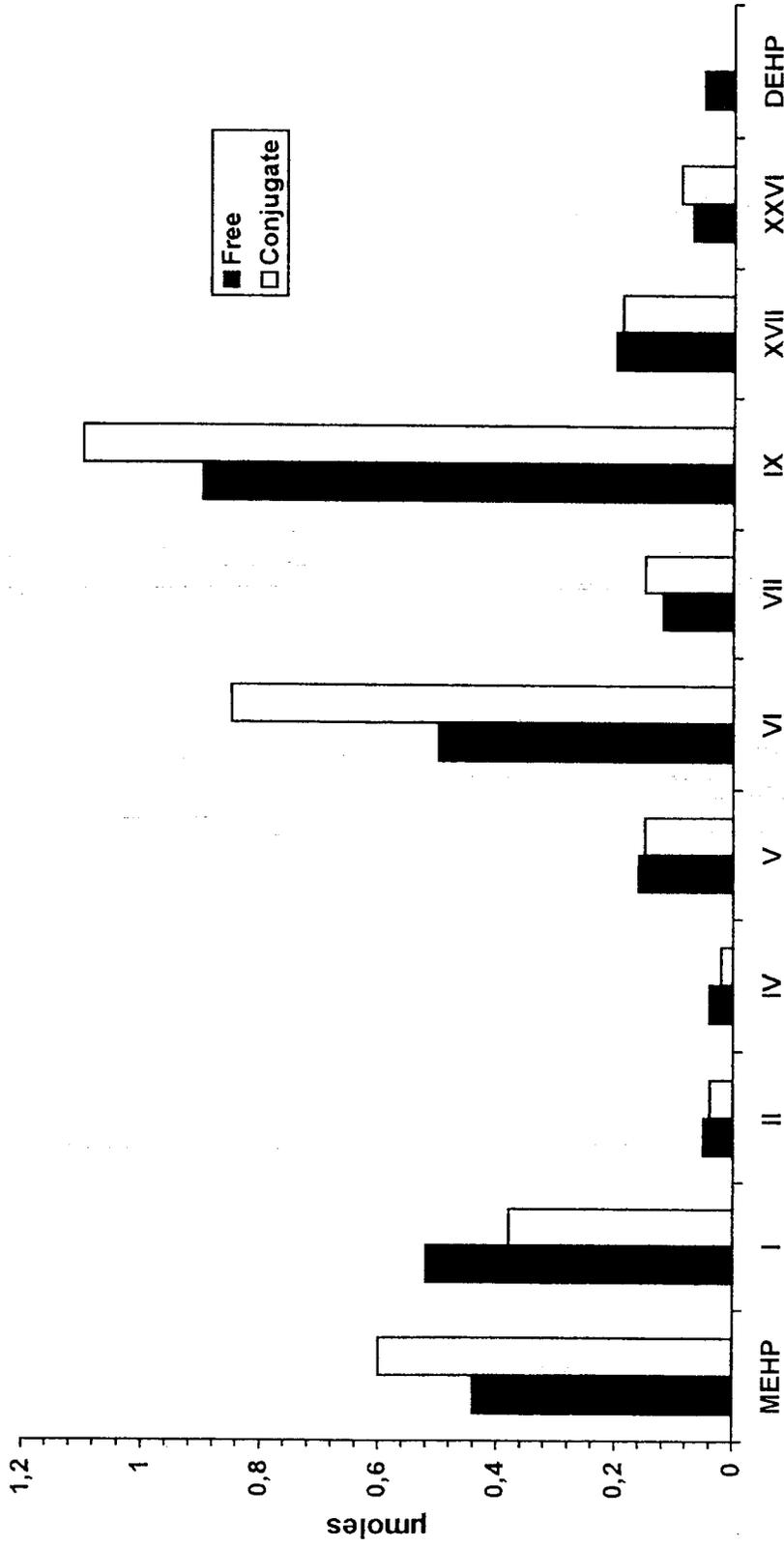


Figure 8. Cumulative excretion (0-72h) and distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice following a single oral administration of 1000 mg/kg DEHP.

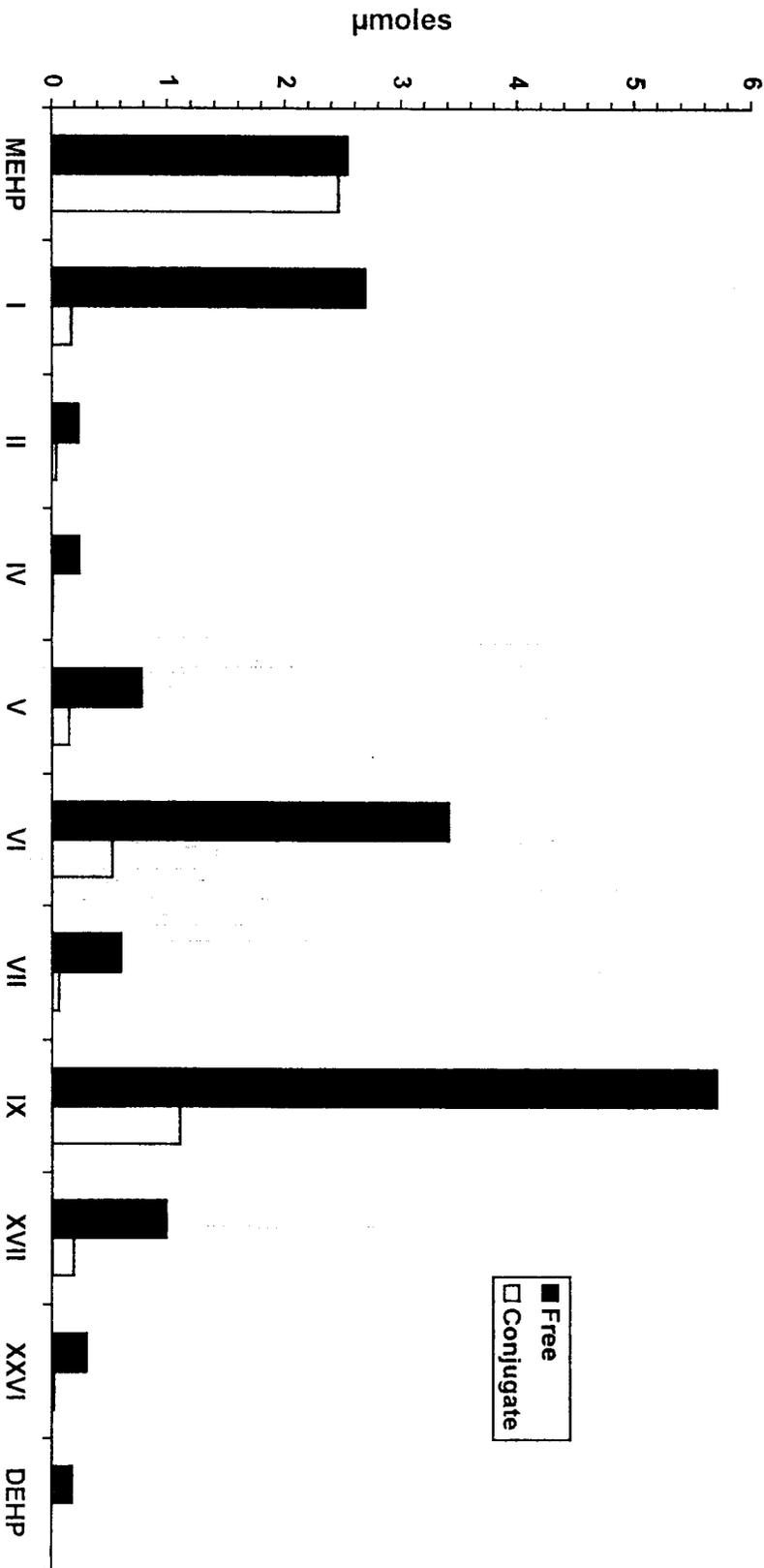


Figure 9. Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

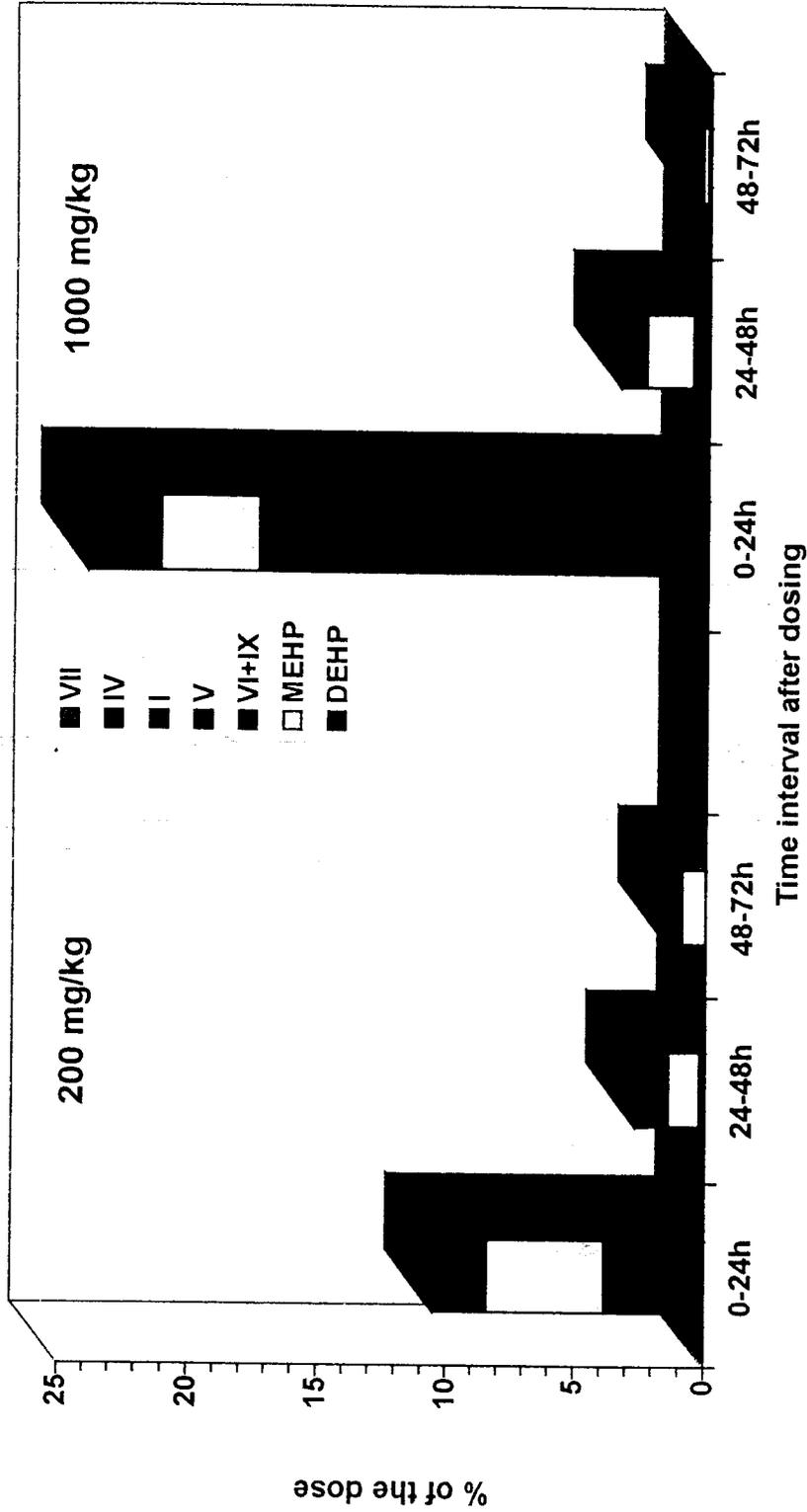


Figure 10. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female mice following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

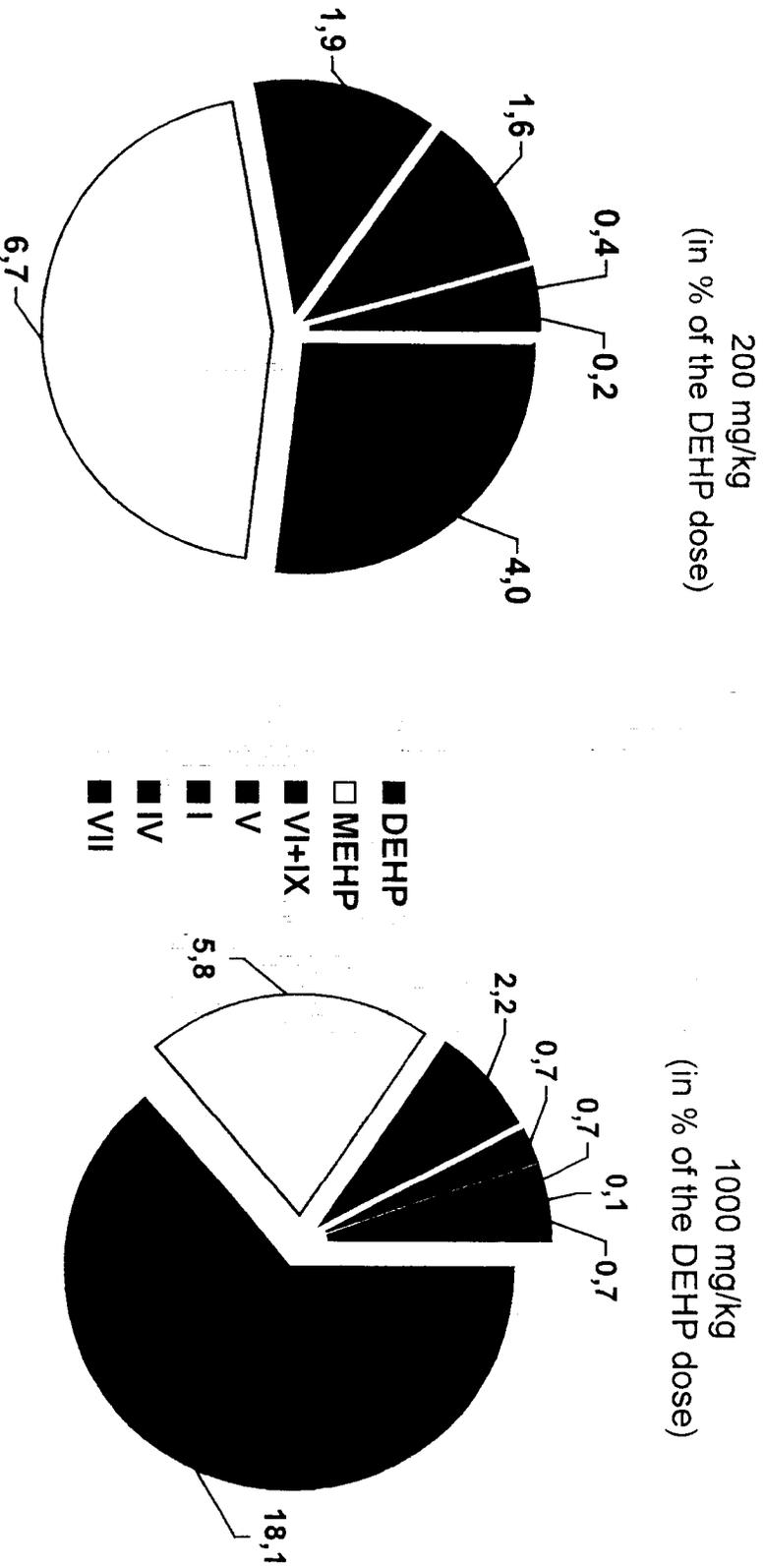
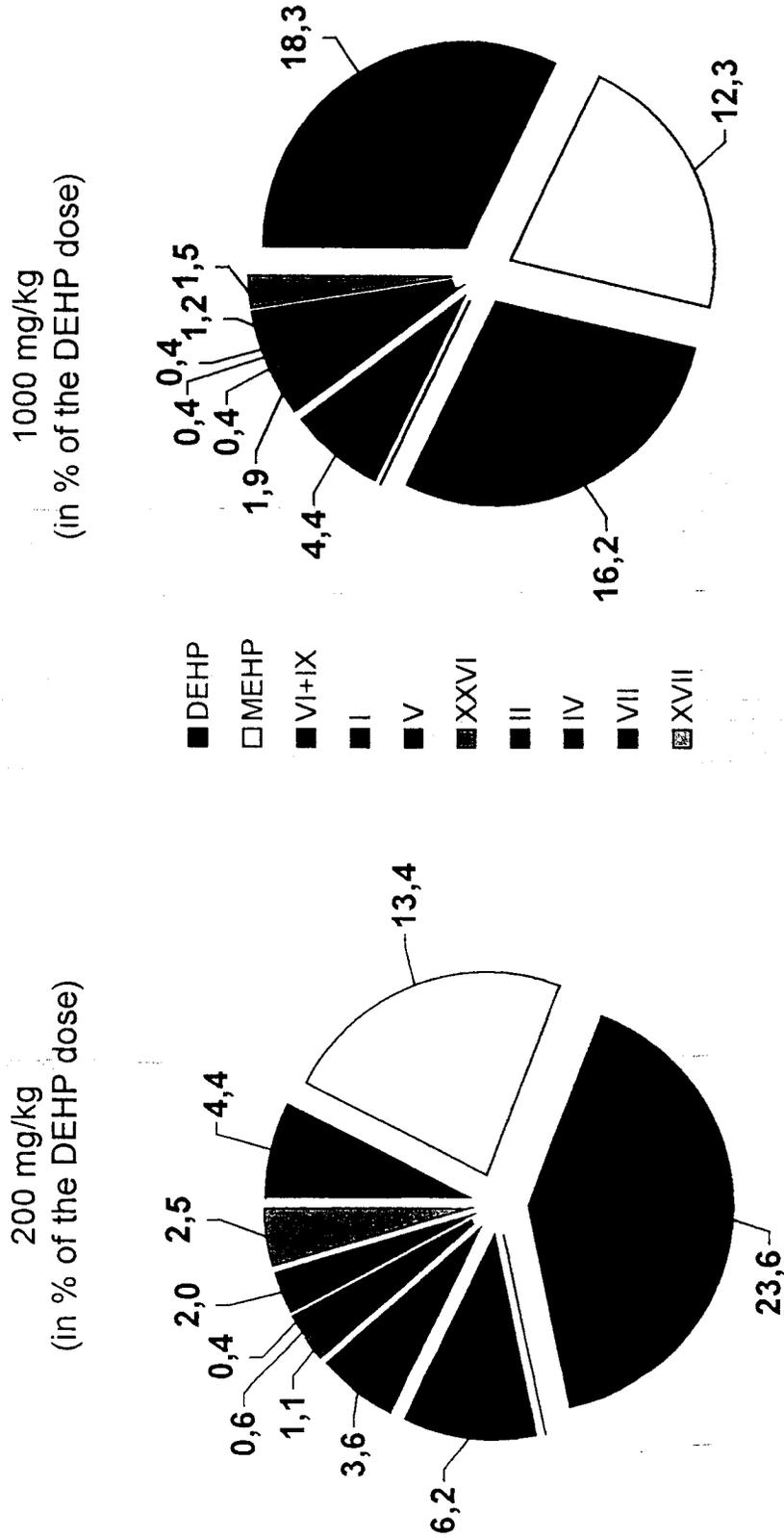


Figure 11. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in the urine and faeces of female mice treated with a single oral dose of 200 mg/kg or 1000 mg/kg DEHP.



Excretion and metabolism study, repeated dosing.

Figure 12. Mean recovery of radioactivity (in $\mu\text{moles DEHP-equivalents}$) in urine and faeces from female mice collected within 24 hours following the 1st (on D0) to the 9th (on D8 included) oral administration of 200 mg/kg/d [¹⁴C]-DEHP. Mice received a 9-day repeated oral administration of 15.4 $\mu\text{moles/animal/d}$ of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

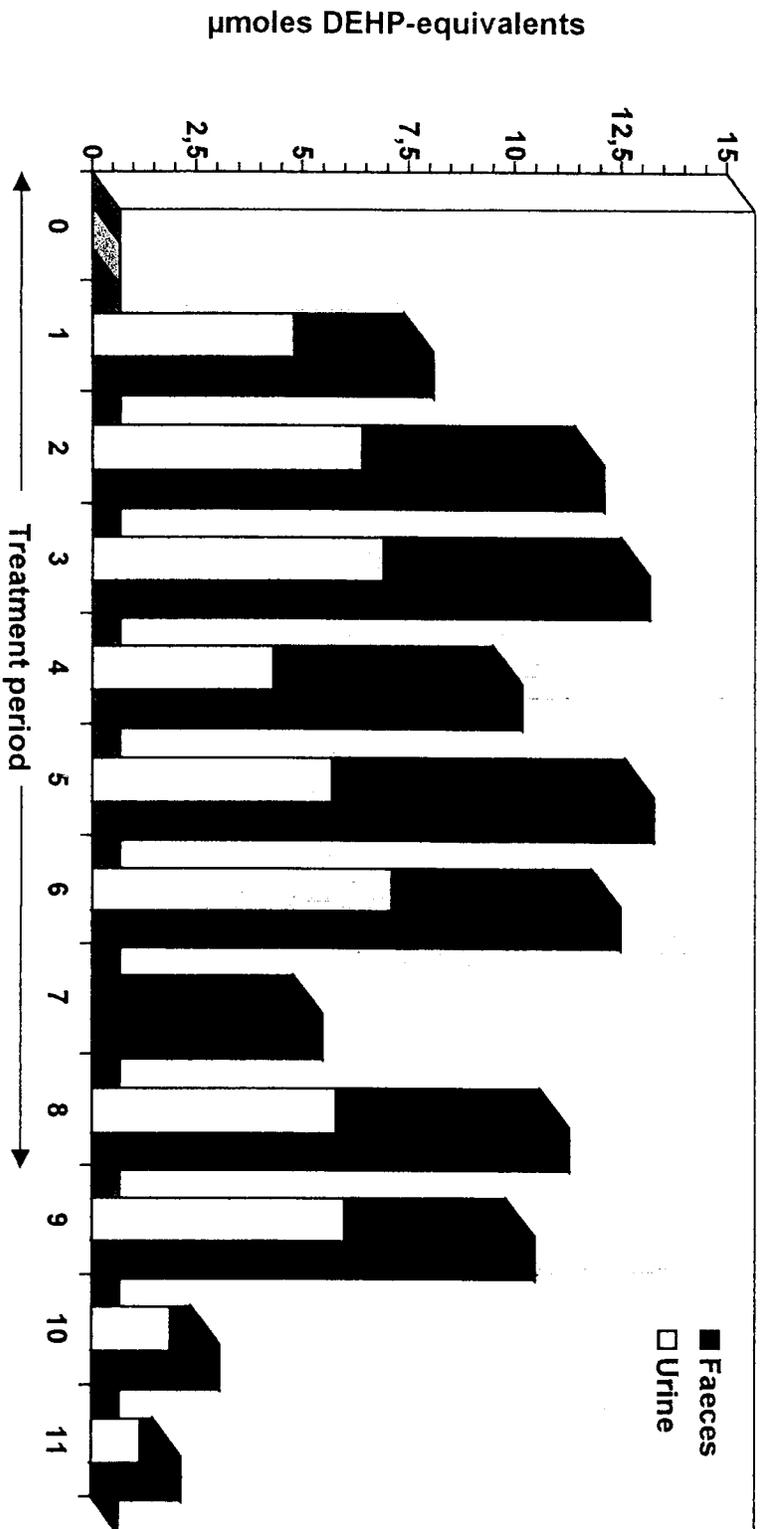


Figure 13. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female mice collected within 24 hours following the 1st (on D0) to the 9th (on D8 included) oral administration of 200 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 15.4 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

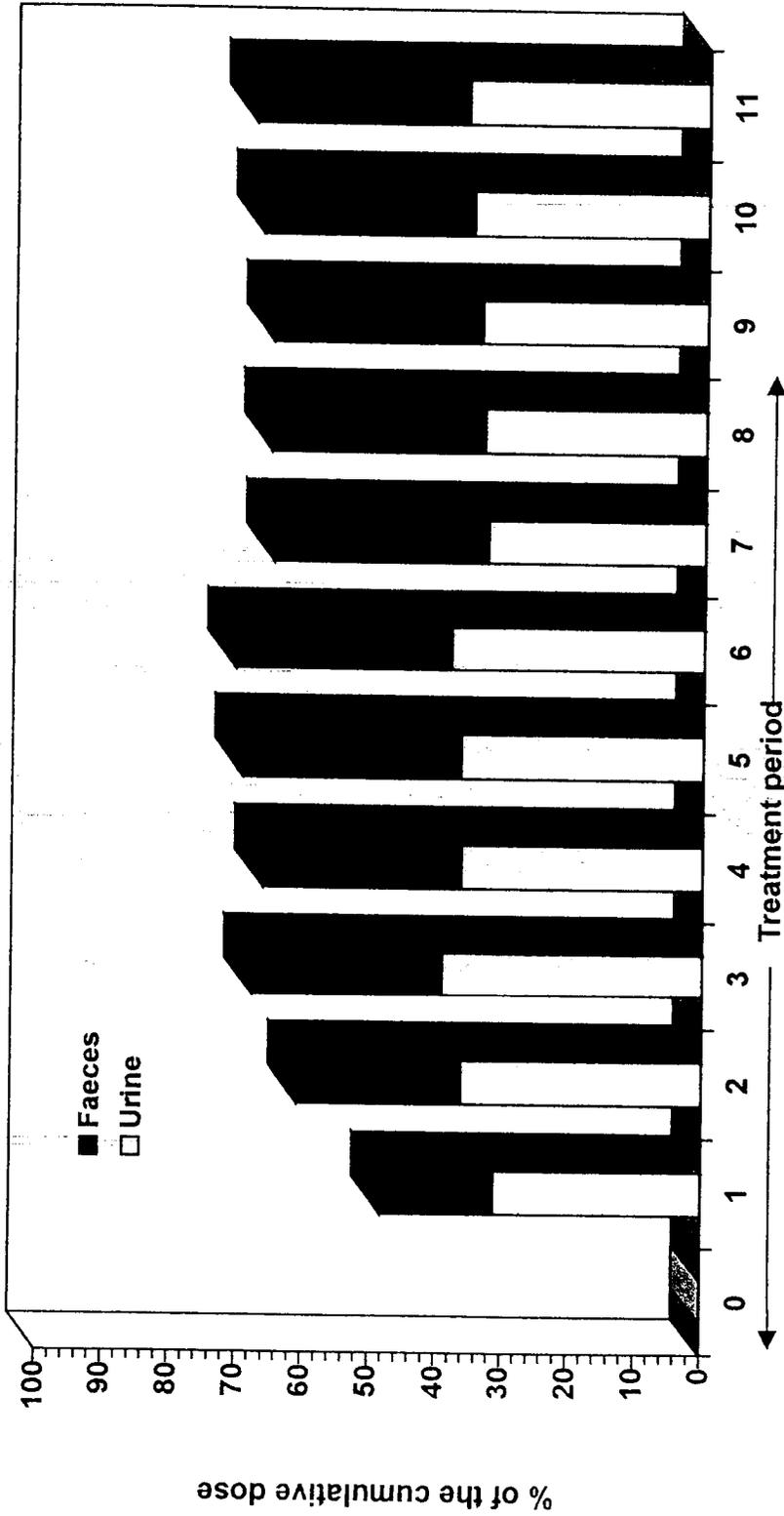


Figure 14. Mean recovery of radioactivity (in μmoles) in urine and faeces from female mice collected within 24 hours following the 1st (on D0) to the 9th (on D8 included) oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 76.9 $\mu\text{moles/animal/d}$ of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

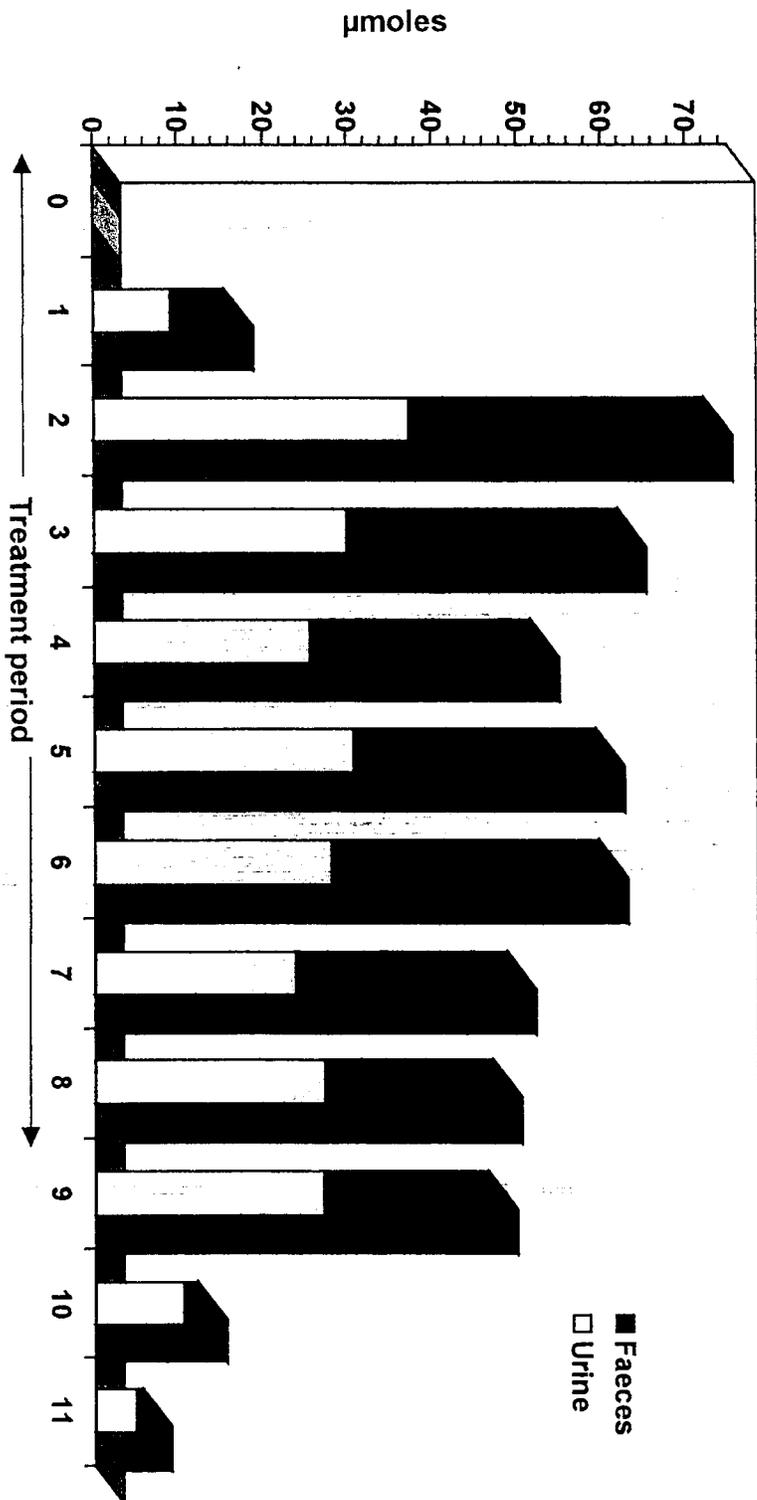


Figure 15. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female mice collected within 24 hours following the 1st (on D0) to the 9th (on D8 included) oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 76.9 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

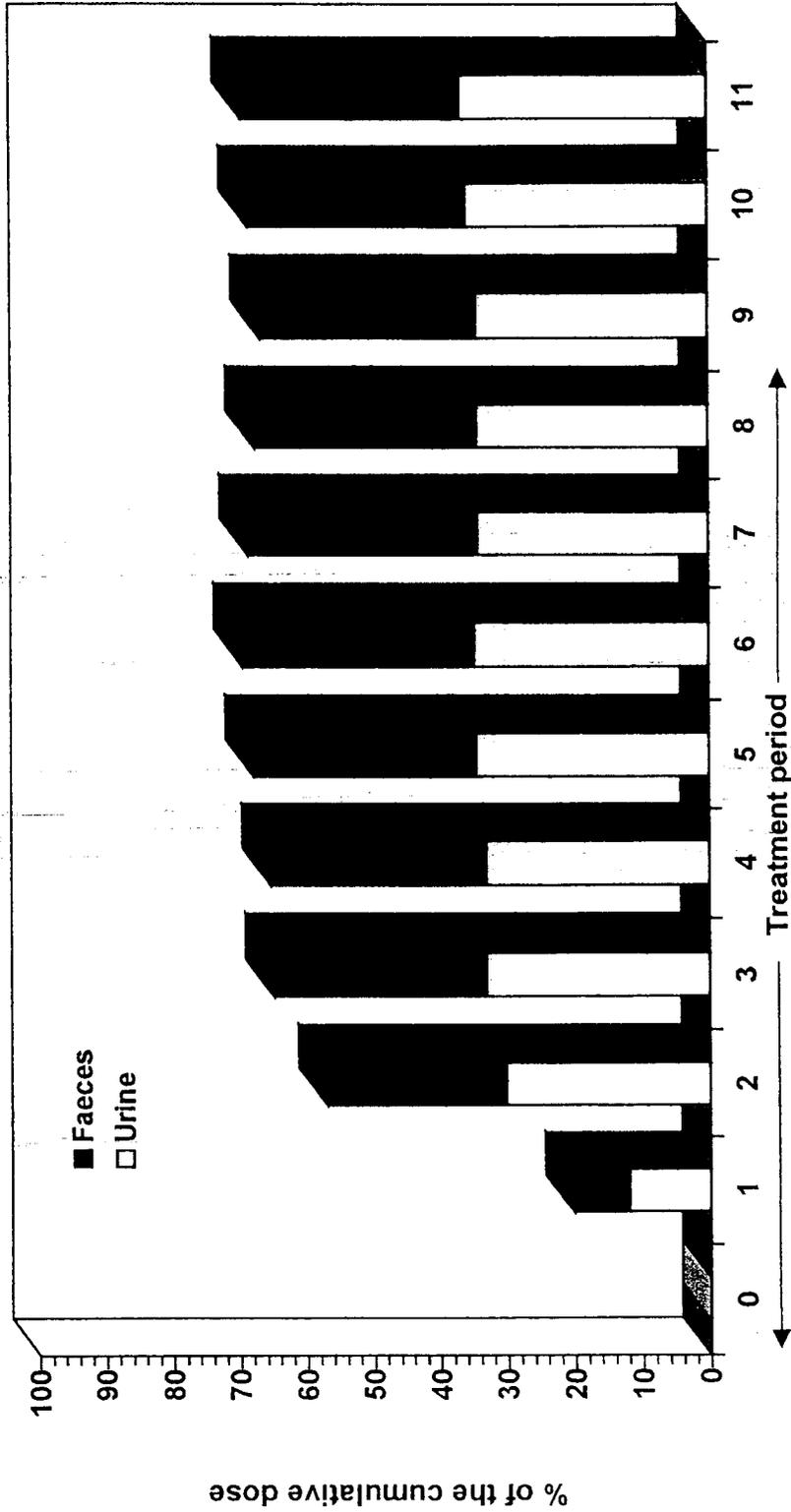


Figure 16. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice within 24 hours following the 1st and 4th oral administration of 200 mg/kg [¹⁴C]-DEHP.

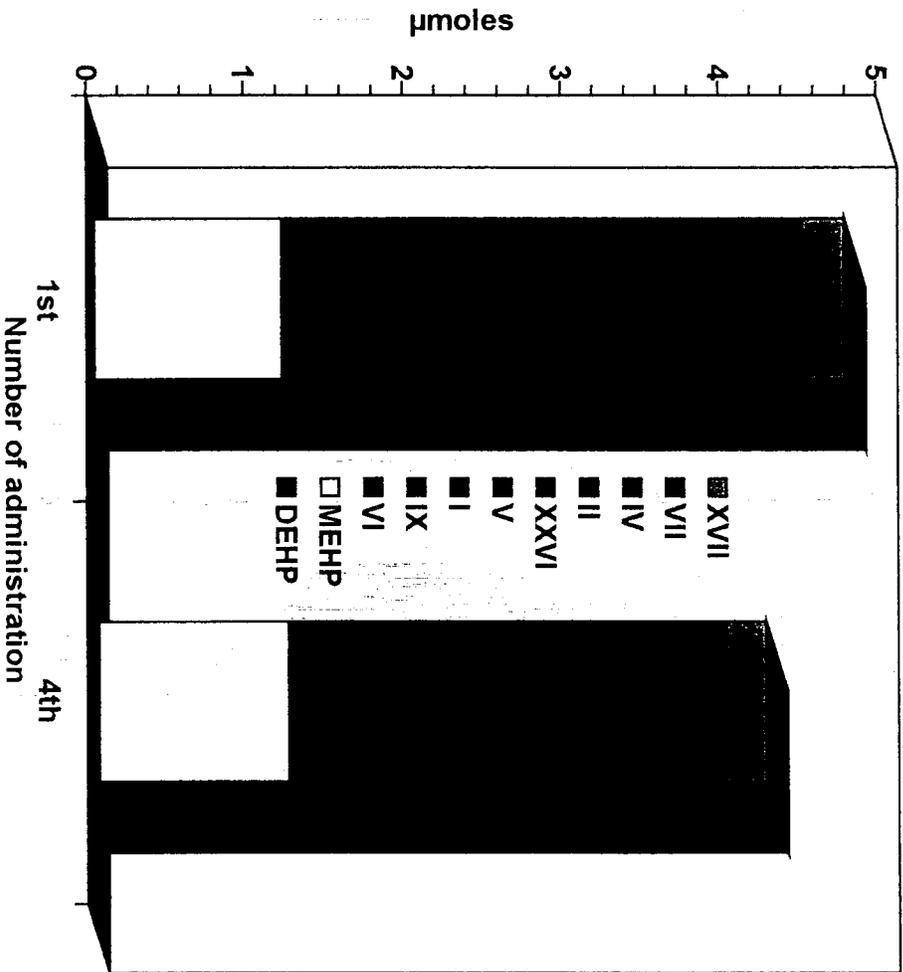
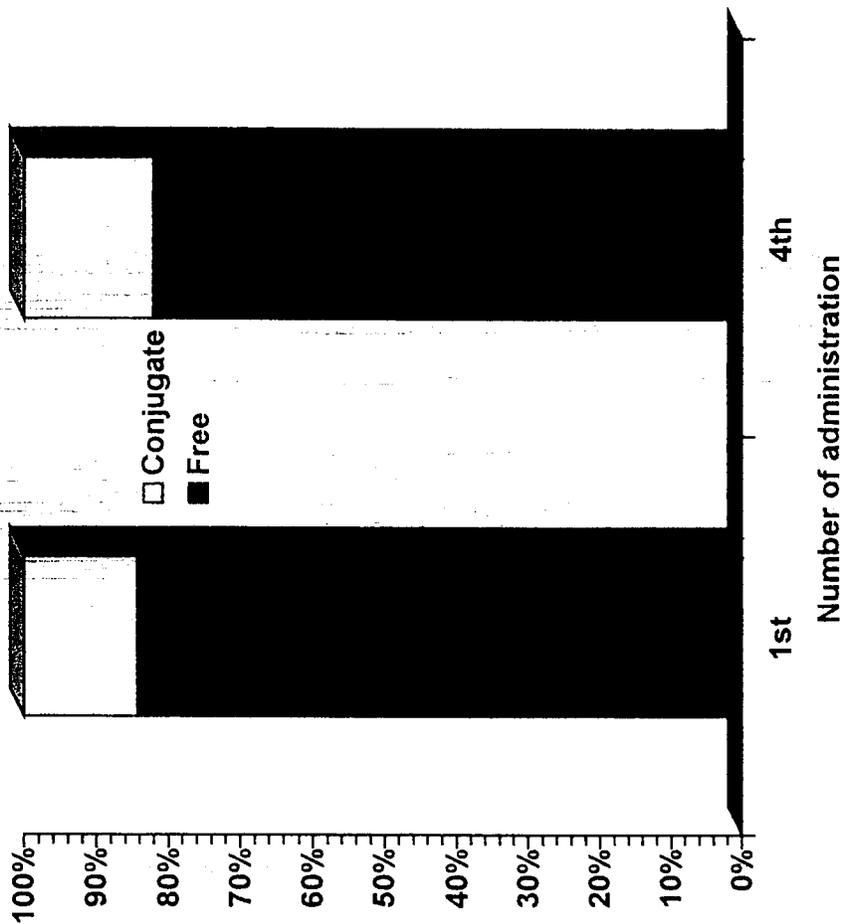


Figure 17. Distribution of free and conjugate metabolites in urine of female mice within 24 hours following the 1st and 4th oral administration of 200 mg/kg [¹⁴C]-DEHP.



48/130

Figure 18. Distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female mice within 24 hours following the 1st and 4th oral administration of 200 mg/kg [¹⁴C]-DEHP.

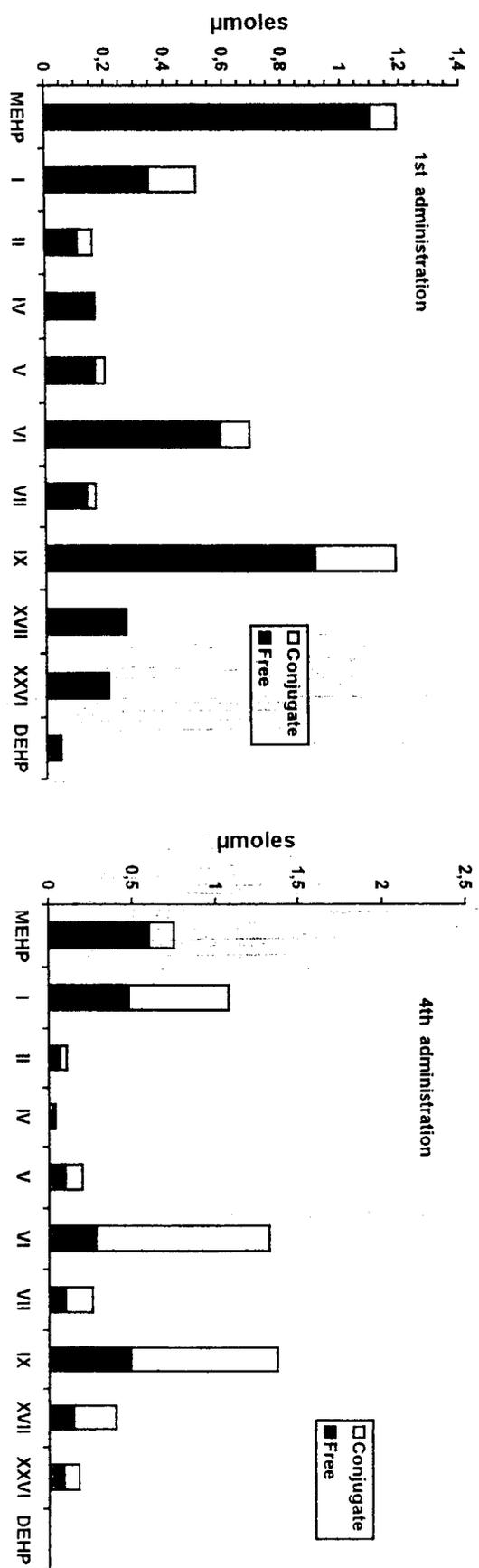


Figure 19. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in faeces from female mice within 24h hours following the 1st, 4th and 7th oral administration of 200 mg/kg [¹⁴C]-DEHP.

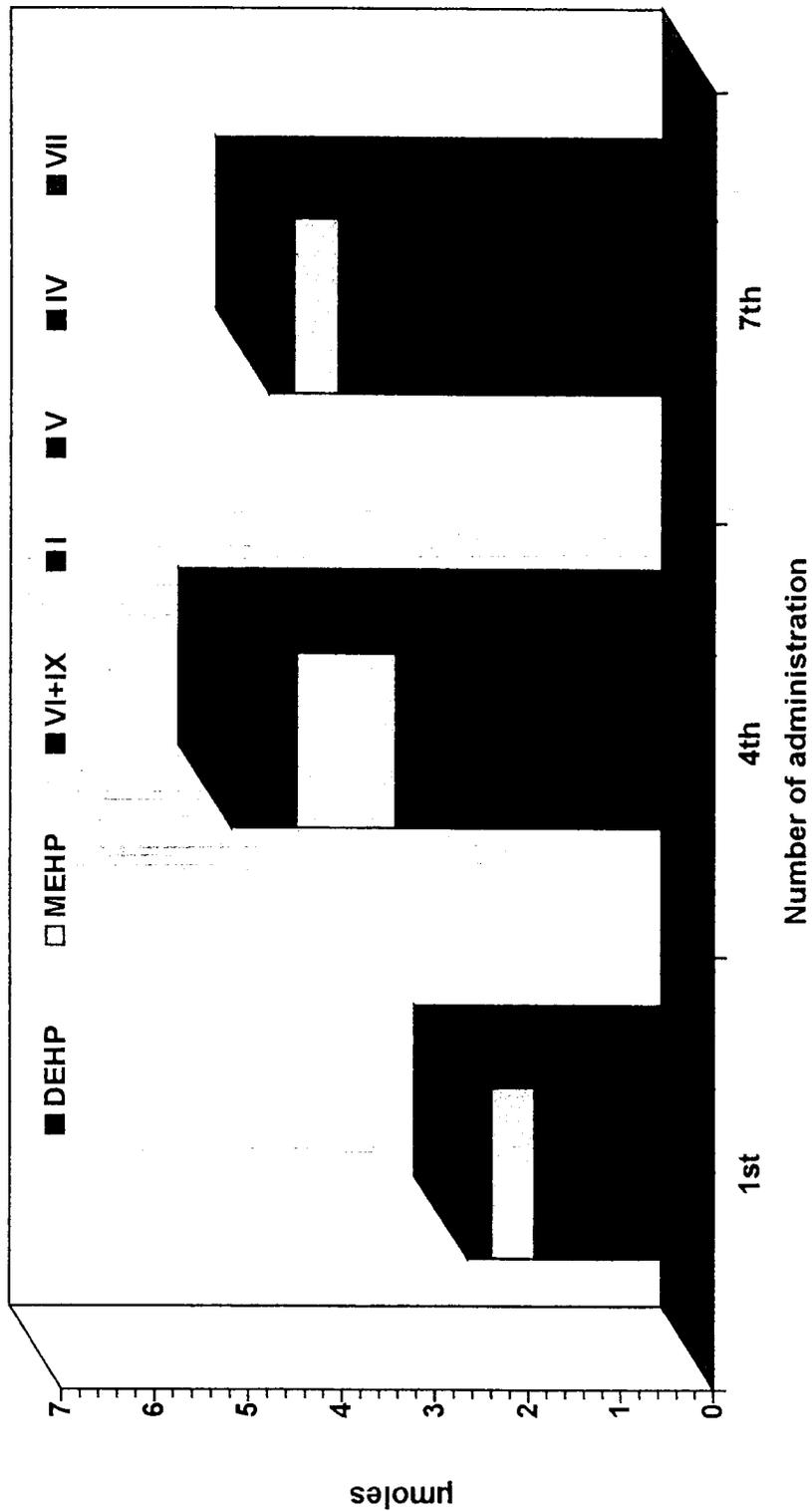


Figure 20. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in the urine and faeces of female mice within 24 hours following the 1st and 4th oral administration of 200 mg/kg [¹⁴C]-DEHP.

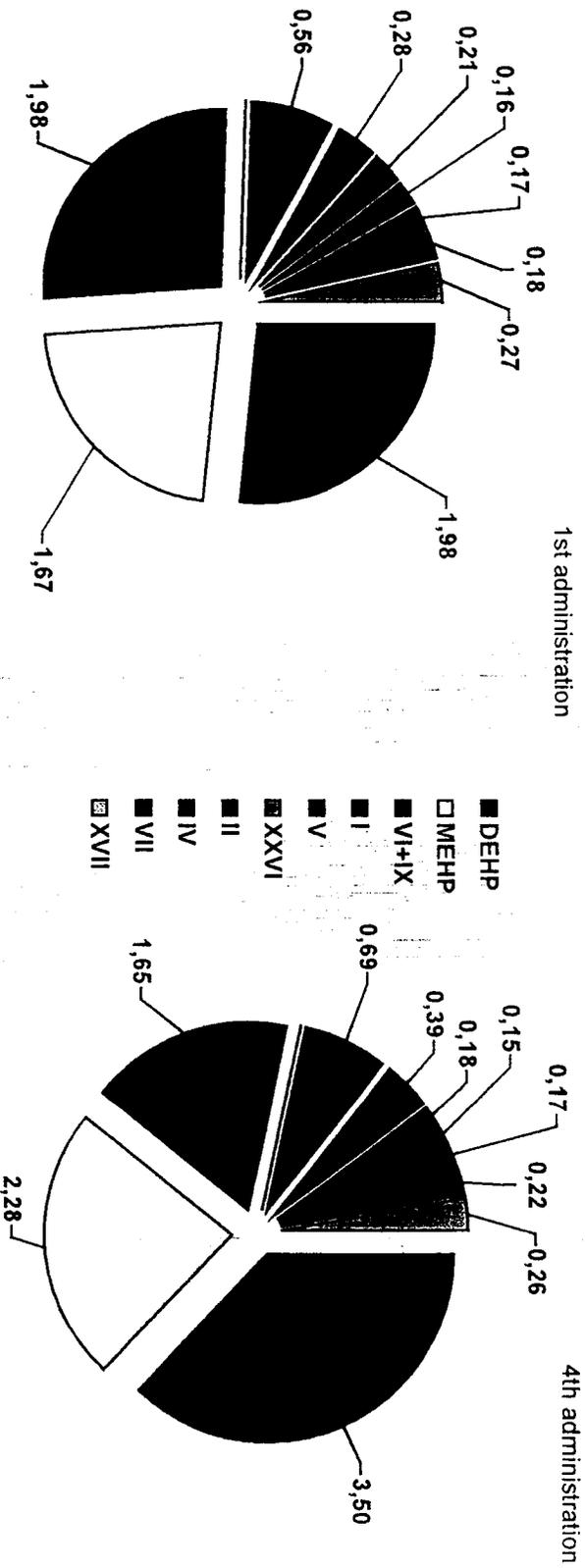


Figure 21. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female mice within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg [^{14}C]-DEHP.

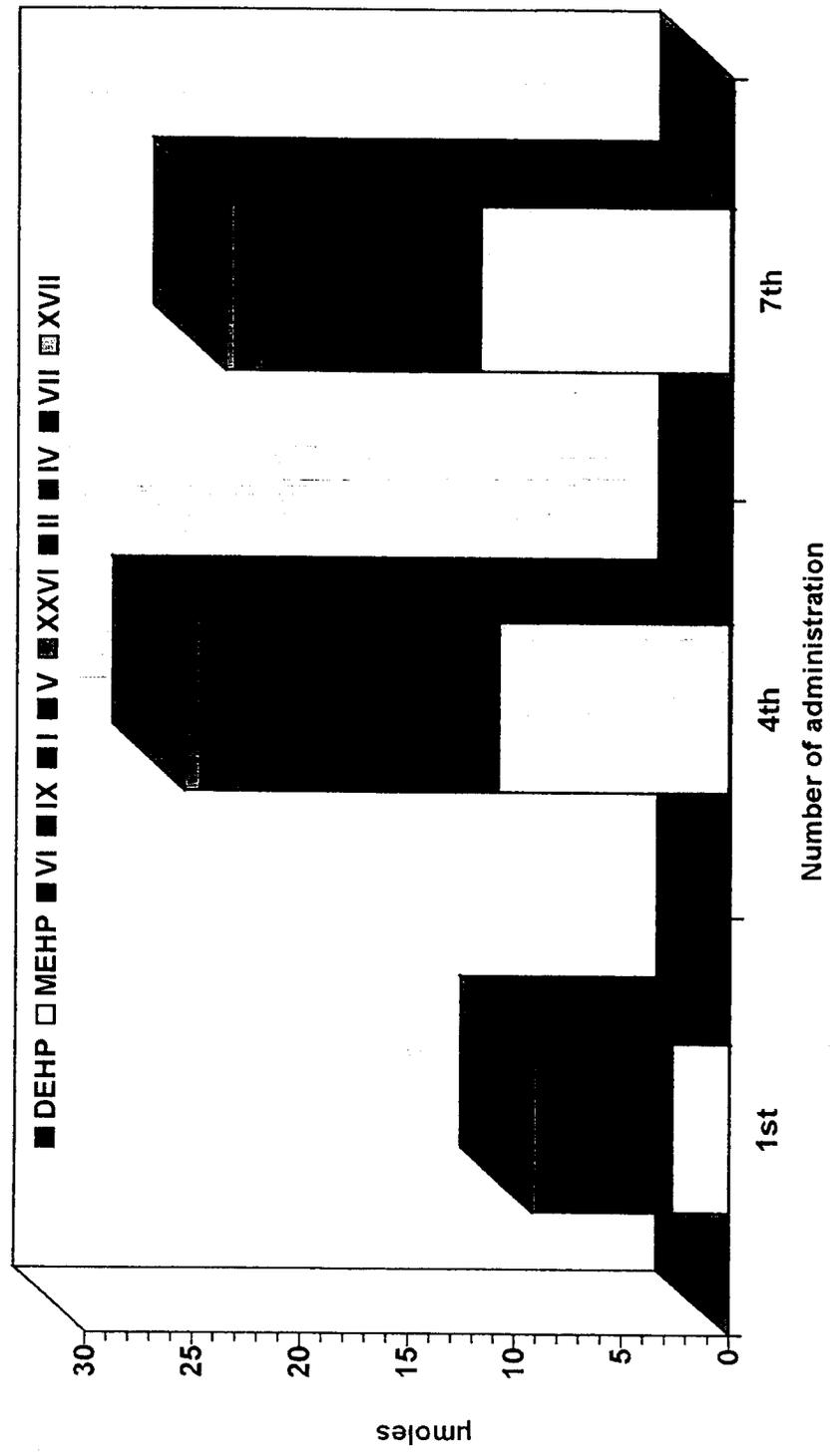


Figure 22. Distribution of free and conjugate metabolites in urine of female mice within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg [¹⁴C]-DEHP.

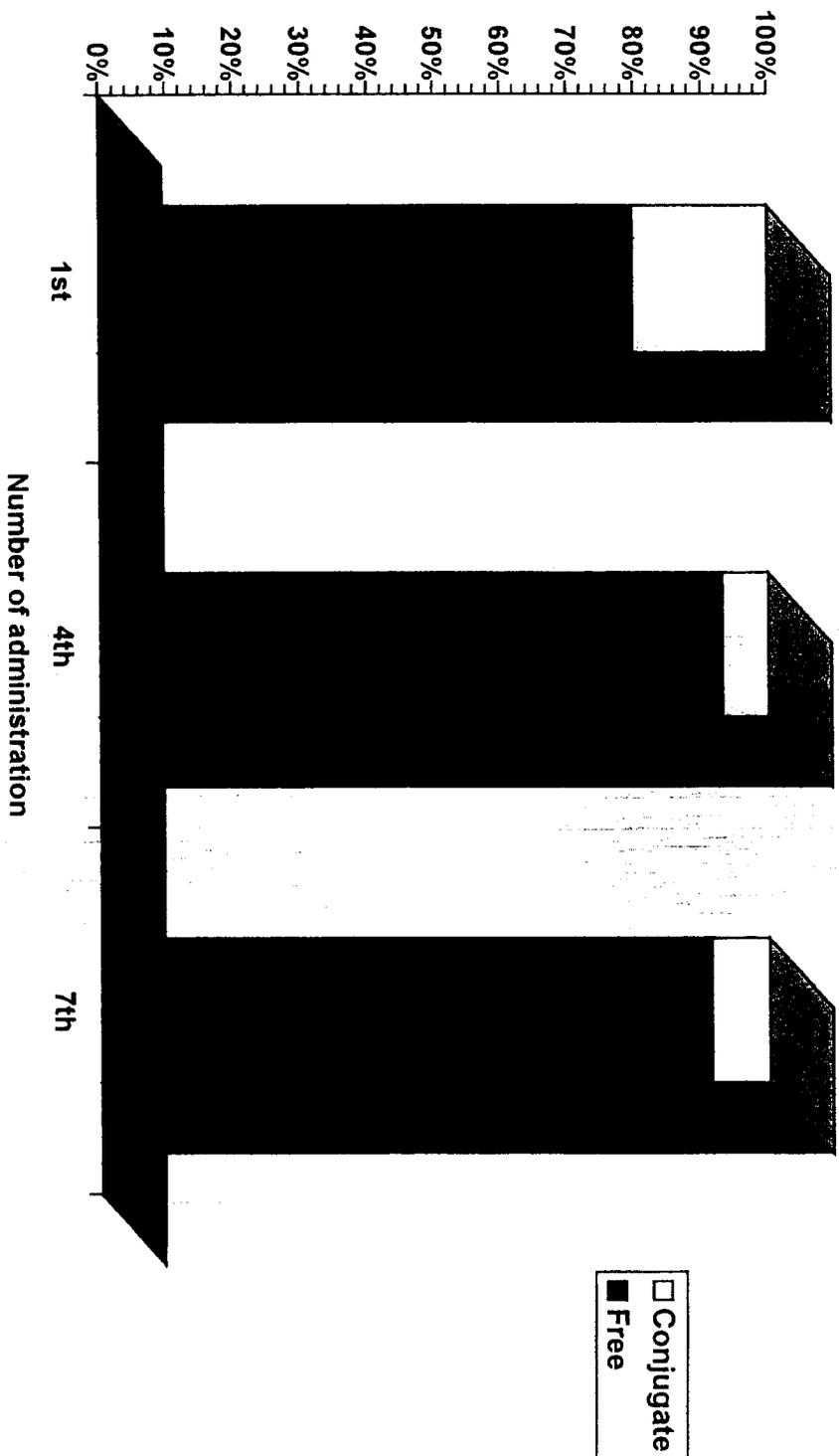


Figure 23. Distribution (free and conjugate) of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female mice within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg [¹⁴C]-DEHP.

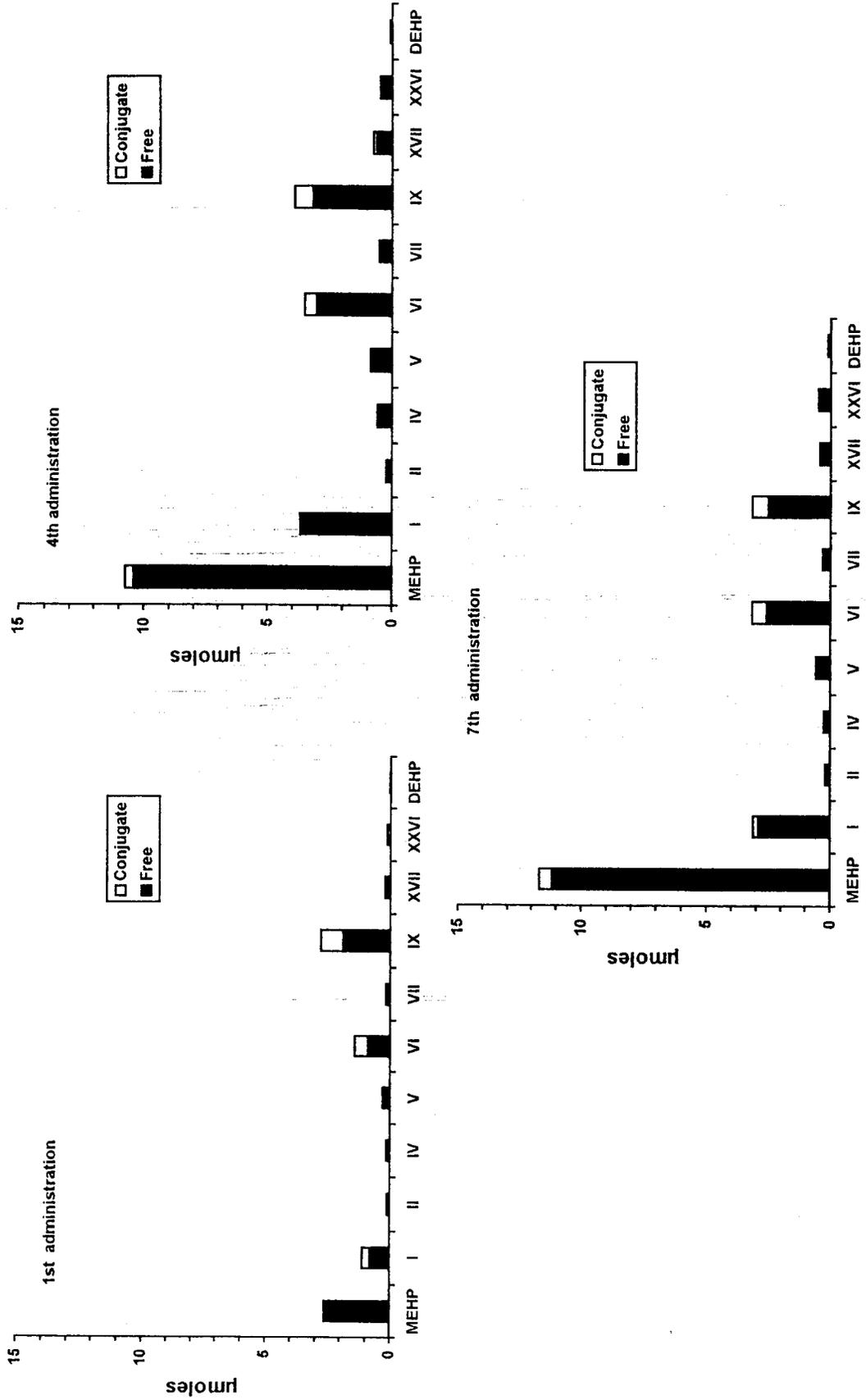


Figure 24. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female mice within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg [¹⁴C]-DEHP.

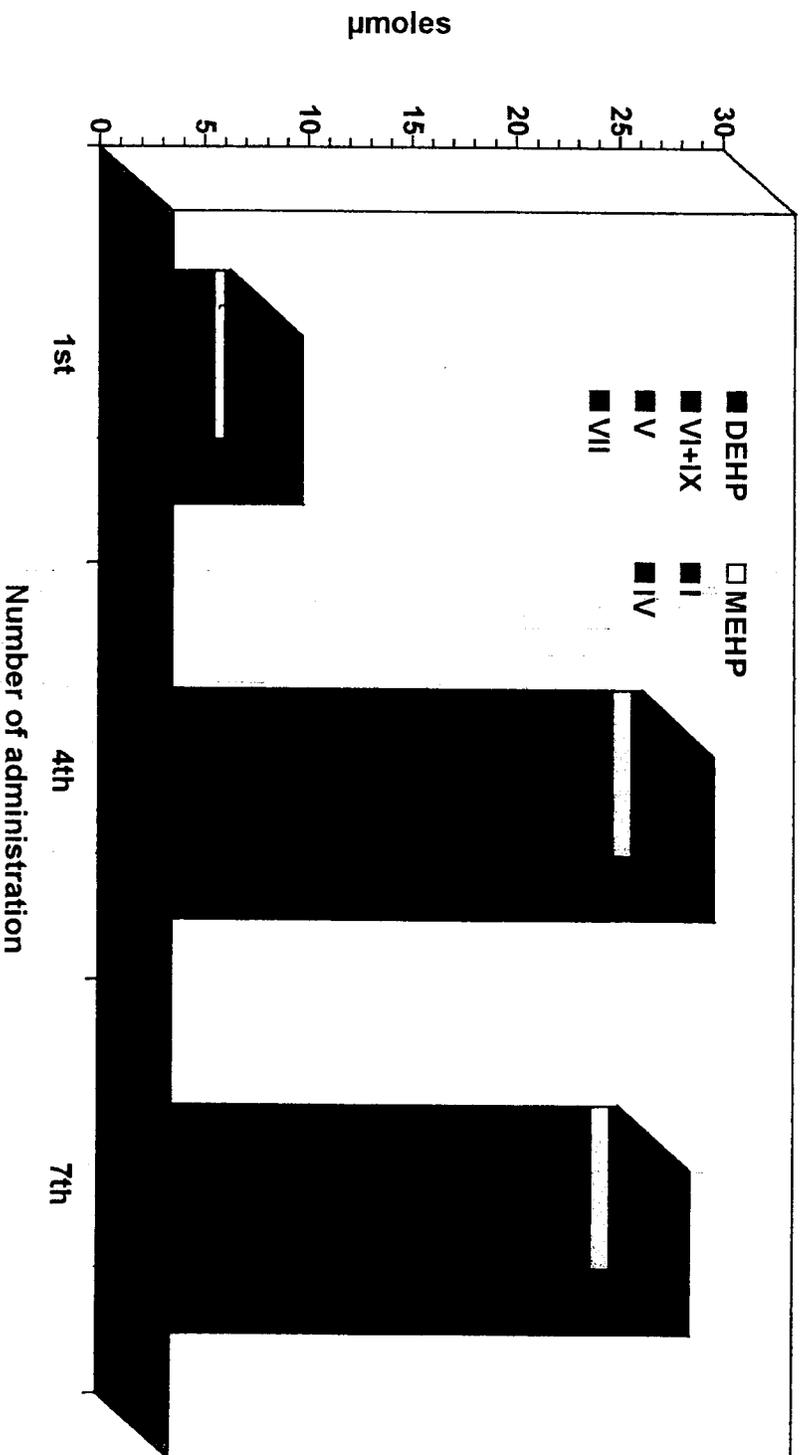
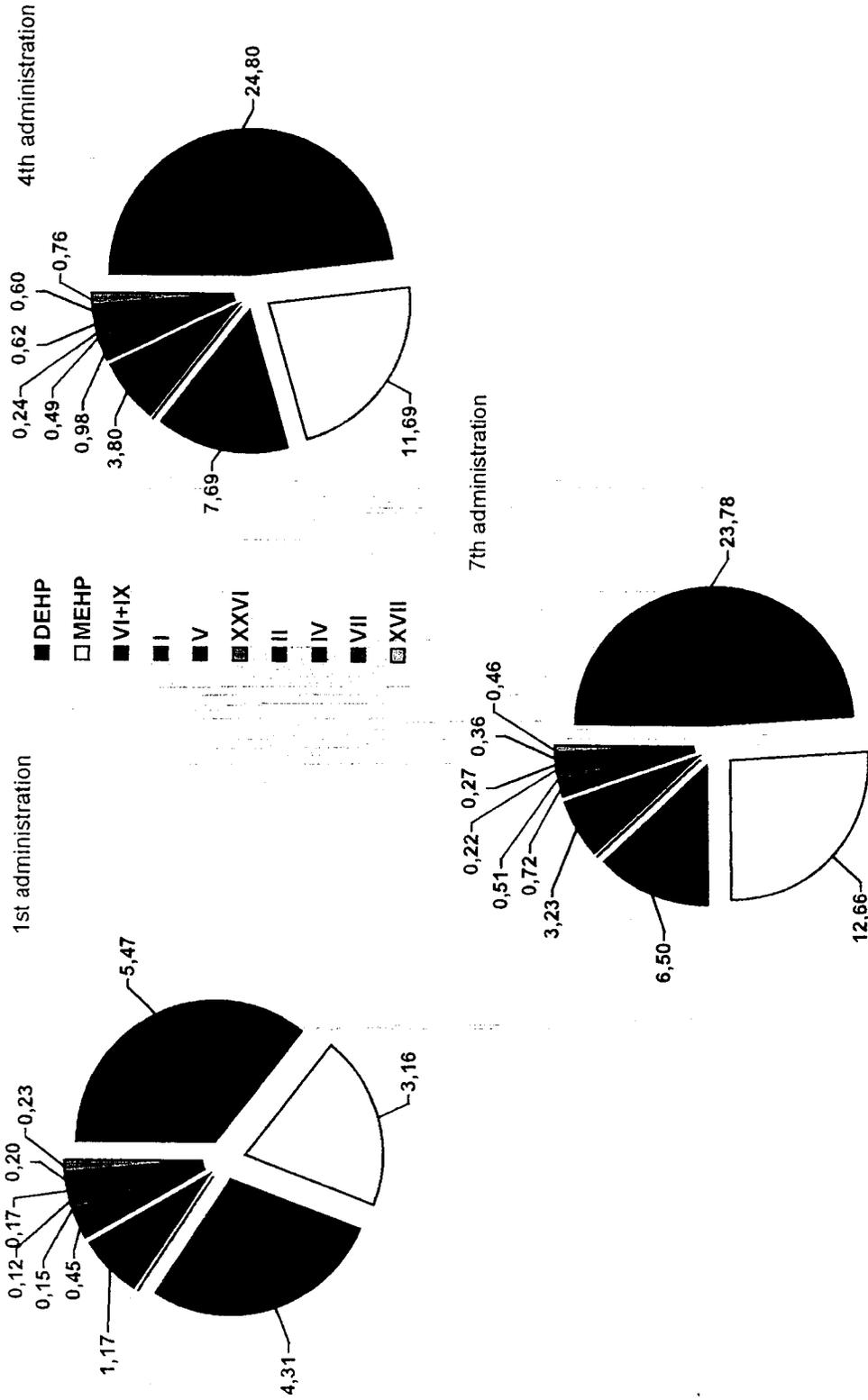


Figure 25. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in the urine and faeces of female mice within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg [¹⁴C]-DEHP.





TABLES

Pharmacokinetic study

Table 1. Mean (and standard deviation) body weight values in female mice treated with a single administration of 200 or 1000 mg/kg DEHP on Day 0.

Day	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
D-5	24.3	1.1	6	24.6	1.2	6
D0	25.3	1.0	6	26.0	1.0	6
D1	25.4	1.4	6	24.7	1.9	6
D2	25.3	1.3	6	24.7	2.4	6

Table 2. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice following a single oral administration of 200 or 1000 mg/kg [¹⁴C]-DEHP.

Time after dosing	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
0.5h	154	17	3	333	150	3
1h	98	38	3	342	84	3
1h30	96	30	3	1339	521	3
4h	110	21	3	271	40	3
24h	25	7	3	58	29	3
48h	1	0.2	3	14	10	3

Table 3. Mean (and standard deviation) body weight values in female mice treated with a 6-day repeated oral administration of 200 or 1000 mg/kg DEHP from D0 to D5 (inclusive).

Day	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
Reception	25.7	1.5	12	24.8	1.8	15
D-1	26.0	1.8	12	26.0	1.7	15
D0	26.3	1.7	12	26.0	1.6	15
D1	26.4	1.6	12	26.2	2.0	15
D2	26.4	1.8	12	26.0	1.6	15
D3	26.4	1.7	12	26.0	1.7	15
D4	26.7	1.7	12	26.4	1.5	15
D5	26.9	2.1	12	26.3	1.5	15
D6	25.8	2.3	12	25.3	1.4	15
D7	26.6	1.7	12	25.5	1.8	15
D8	26.6	1.7	12	25.7	1.8	15

Table 4. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g blood) from female mice after a single administration 200 or 1000 mg/kg of [¹⁴C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled DEHP, respectively.

Time after dosing	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
0.5h	85	18	3	178	43	3
1h	117	42	3	329	128	3
1h30	197	60	3	301	47	2
4h	155	63	3	396	66	3
24h	4	1	3	20	17	3
48h	2	2	3	6	3	3

Excretion and metabolism study, single dosing**Table 5. Mean (and standard deviation) body weight values in control female mice and in female mice treated with a single administration of 200 or 1000 mg/kg [¹⁴C]-DEHP on Day 0.**

Day	Control			200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
D-4	25.8	0.9	4	25.4	0.5	6	24.7	1.2	6
D0	25.6	1.4	4	25.7	1.4	6	24.7	1.2	6
D1	24.8	2.0	4	24.4	1.5	6	24.0	1.8	6
D2	26.1	2.5	4	24.6	1.8	6	25.2	1.7	6
D3	26.6	1.9	4	25.4	1.8	6	25.9	1.7	6
D4	26.6	1.8	4	25.9	1.4	6	25.8	1.2	6

Table 6. Recovery of radioactivity (as percentage of the [¹⁴C]-DEHP dose and as μmole DEHP-equivalents) in urine and faeces from female mice following a single oral administration of 200 mg/kg.

Time interval	% of the dose						μmole DEHP-equivalents									
	Urine			Faeces			Total		Urine			Faeces			Total	
	Mean	SD	n	Mean	SD	n	Mean	SD	Mean	SD	n	Mean	SD	n	Mean	SD
0-24 h	30.9	6.9	3	10.5	5.6	3	41.4	8.8	4.7	1.1	3	1.6	0.9	3	6.3	1.3
24-48 h	9.2	5.5	3	2.7	0.9	3	11.9	4.6	1.4	0.8	3	0.4	0.2	3	1.8	0.7
48-72 h	3.0	1.5	3	1.5	0.4	3	4.5	1.7	0.4	0.3	3	0.2	0.1	3	0.7	0.3
72-96 h	1.3	0.6	3	0.6	0.5	3	1.9	1.1	0.2	0.1	3	0.1	0.1	3	0.3	0.2
Total	44.4	10.4	3	15.4	6.2	3	59.7	7.0	6.8	1.6	3	2.4	0.9	3	9.1	1.0

Table 7. Recovery of radioactivity (as percentage of the [¹⁴C]-DEHP dose and as μmole DEHP-equivalents) in urine and faeces from female mice following a single oral administration of 1000 mg/kg.

Time interval	% of the dose						μmole DEHP-equivalents									
	Urine			Faeces			Total		Urine			Faeces			Total	
	Mean	SD	n	Mean	SD	n	Mean	SD	Mean	SD	n	Mean	SD	n	Mean	SD
0-24 h	18.4	3.8	3	23.9	9.2	3	42.3	12.1	14.2	3.0	3	18.4	7.1	3	32.5	9.3
24-48 h	7.9	2.3	3	3.3	2.0	3	11.2	4.2	6.1	1.8	3	2.6	1.5	3	8.6	3.2
48-72 h	2.8	0.5	3	0.6	0.3	3	3.5	0.8	2.2	0.4	3	0.5	0.2	3	2.7	0.6
72-96 h	1.8	0.3	3	0.3	0.1	3	2.1	0.4	1.4	0.3	3	0.2	0.1	3	1.7	0.3
Total	31.0	5.5	3	28.2	7.5	3	59.2	11.1	23.8	4.2	3	21.7	5.8	3	45.5	8.6

Table 8 (continued)

Metabolites	µmoles												% of the dose											
	0-24 h			24-48 h			48-72 h			Total			0-24 h			24-48 h			48-72 h			Total		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	0.68	0.18	3	0.24	0.11	3	0.11	0.05	3	1.03	0.34		4.4	1.2	3	1.5	0.7	3	0.7	0.3	3	6.7	2.2	
I	0.59	0.13	3	0.25	0.14	3	0.05	0.03	3	0.89	0.30		3.9	0.9	3	1.6	0.9	3	0.3	0.2	3	5.8	1.9	
II	0.06	0.01	3	0.02	0.01	3	0.01	0.00	3	0.09	0.03		0.4	0.1	3	0.1	0.1	3	0.0	0.0	3	0.6	0.2	
IV	0.03	0.00	3	0.02	0.02	3	0.00	0.00	3	0.06	0.03		0.2	0.0	3	0.1	0.1	3	0.0	0.0	3	0.4	0.2	
V	0.22	0.04	3	0.07	0.03	3	0.02	0.01	3	0.31	0.08		1.4	0.3	3	0.4	0.2	3	0.2	0.0	3	2.0	0.5	
VI	1.04	0.22	3	0.24	0.16	3	0.06	0.05	3	1.34	0.42		6.8	1.4	3	1.5	1.0	3	0.4	0.3	3	8.7	2.7	
VII	0.19	0.05	3	0.06	0.04	3	0.02	0.02	3	0.27	0.10		1.3	0.3	3	0.4	0.3	3	0.1	0.1	3	1.8	0.7	
IX	1.48	0.35	3	0.41	0.29	3	0.11	0.10	3	2.00	0.74		9.6	2.3	3	2.7	1.9	3	0.7	0.7	3	13.0	4.8	
XVII	0.28	0.06	3	0.08	0.04	3	0.03	0.01	3	0.39	0.10		1.8	0.4	3	0.5	0.2	3	0.2	0.1	3	2.5	0.7	
XXVI	0.12	0.03	3	0.03	0.02	3	0.01	0.01	3	0.16	0.05		0.8	0.2	3	0.2	0.1	3	0.1	0.0	3	1.1	0.3	
DEHP	0.04	0.01	3	0.01	0.00	3	0.00	0.00	3	0.06	0.02		0.3	0.1	3	0.1	0.0	3	0.0	0.0	3	0.4	0.1	
Total	4.75	1.03	3	1.42	0.83	3	0.43	0.25	3	6.60	2.11		30.8	6.7	3	9.2	5.4	3	2.8	1.6	3	42.8	13.7	

T O T A L

Table 9. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (expressed as μ moles or as % of the DEHP dose) excreted in faeces from female mice following a single oral administration of 200 mg/kg.

Metabolites	μ moles												% of the dose											
	0-24 h			24-48 h			48-72 h			Total			0-24 h			24-48 h			48-72 h			Total		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	0.71	0.25	3	0.19	0.11	3	0.14	0.03	3	1.03	0.39		4.6	1.6	3	1.2	0.7	3	0.9	0.2	3	6.7	2.6	
I	0.07	0.05	3	trace	-	3	trace	-	3	0.07	0.05		0.4	0.3	3	trace	-	3	trace	-	3	0.4	0.3	
IV	0.00	0.00	3	0.00	0.00	3	0.00	0.00	3	0.00	0.00		0.0	0.0	3	0.0	0.0	3	0.0	0.0	3	0.0	0.0	
V	0.13	0.05	3	0.08	0.01	3	0.04	0.01	3	0.25	0.06		0.9	0.3	3	0.5	0.1	3	0.2	0.0	3	1.6	0.4	
VII	0.02	0.02	3	0.00	0.00	3	0.01	0.01	3	0.03	0.03		0.1	0.1	3	0.0	0.0	3	0.0	0.1	3	0.2	0.2	
VI + IX	0.10	0.04	3	0.13	0.04	3	0.06	0.01	3	0.29	0.10		0.7	0.3	3	0.8	0.3	3	0.4	0.1	3	1.9	0.7	
DEHP	0.58	0.45	3	0.03	0.01	3	0.01	0.00	3	0.62	0.46		3.8	2.9	3	0.2	0.1	3	0.0	0.0	3	4.0	3.0	
Total	1.62	0.85	3	0.43	0.15	3	0.25	0.05	3	2.29	1.05		10.5	5.5	3	2.8	1.0	3	1.6	0.3	3	14.9	6.8	

Table 10 (continued)

Metabolites	µmoles											
	0-24 h			24-48 h			48-72 h			Total		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	2.99	1.24	3	1.55	0.21	3	0.46	0.04	3	5.00	1.50	
I	1.60	0.53	3	0.95	0.44	3	0.31	0.09	3	2.86	1.07	
II	0.19	0.03	3	0.06	0.02	3	0.02	0.01	3	0.27	0.06	
IV	0.18	0.06	3	0.04	0.02	3	0.03	0.01	3	0.25	0.08	
V	0.63	0.08	3	0.21	0.06	3	0.09	0.02	3	0.93	0.15	
VI	2.56	0.88	3	1.00	0.46	3	0.38	0.08	3	3.94	1.42	
VII	0.41	0.08	3	0.17	0.05	3	0.09	0.02	3	0.67	0.15	
IX	4.48	1.48	3	1.72	0.46	3	0.61	0.06	3	6.81	2.01	
XVII	0.79	0.10	3	0.27	0.07	3	0.11	0.02	3	1.18	0.19	
XXVI	0.21	0.05	3	0.07	0.02	3	0.04	0.01	3	0.33	0.09	
DEHP	0.12	0.02	3	0.03	0.01	3	0.02	0.01	3	0.18	0.04	
Total	14.17	2.97	3	6.07	1.76	3	2.17	0.35	3	22.40	5.08	

Metabolites	% of the dose											
	0-24 h			24-48 h			48-72 h			Total		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	3.9	1.6	3	2.0	0.3	3	0.6	0.1	3	6.5	1.9	
I	2.1	0.7	3	1.2	0.6	3	0.4	0.1	3	3.7	1.4	
II	0.2	0.0	3	0.1	0.0	3	0.0	0.0	3	0.4	0.1	
IV	0.2	0.1	3	0.0	0.0	3	0.0	0.0	3	0.3	0.1	
V	0.8	0.1	3	0.3	0.1	3	0.1	0.0	3	1.2	0.2	
VI	3.3	1.1	3	1.3	0.6	3	0.5	0.1	3	5.1	1.8	
VII	0.5	0.1	3	0.2	0.1	3	0.1	0.0	3	0.9	0.2	
IX	5.8	1.9	3	2.2	0.6	3	0.8	0.1	3	8.9	2.6	
XVII	1.0	0.1	3	0.3	0.1	3	0.1	0.0	3	1.5	0.2	
XXVI	0.3	0.1	3	0.1	0.0	3	0.1	0.0	3	0.4	0.1	
DEHP	0.2	0.0	3	0.0	0.0	3	0.0	0.0	3	0.2	0.1	
Total	18.4	3.9	3	7.9	2.3	3	2.8	0.5	3	29.1	6.6	

Table 11. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (expressed as µmoles or as % of the DEHP dose) excreted in faeces from female mice following a single oral administration of 1000 mg/kg.

Table 11. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (expressed as μ moles or as % of the DEHP dose) excreted in faeces from female mice following a single oral administration of 1000 mg/kg.

Metabolites	μ moles												% of the dose													
	0-24 h			24-48 h			48-72 h			Total			0-24 h			24-48 h			48-72 h			Total				
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n		
MEHP	2.93	1.64	3	1.38	1.08	3	0.17	0.09	3	4.47	2.81					3.8	2.1	3	1.8	1.4	3	0.2	0.1	3	5.8	3.7
I	0.38	0.52	3	0.12	0.06	3	0.05	0.05	3	0.55	0.63					0.5	0.7	3	0.2	0.1	3	0.1	0.1	3	0.7	0.8
IV	0.04	0.01	3	0.00	0.00	3	0.01	0.03	3	0.06	0.04					0.1	0.0	3	0.0	0.0	3	0.0	0.0	3	0.1	0.0
V	0.28	0.13	3	0.20	0.07	3	0.07	0.02	3	0.55	0.22					0.4	0.2	3	0.3	0.1	3	0.1	0.0	3	0.7	0.3
VII	0.12	0.16	3	0.07	0.07	3	0.03	0.05	3	0.22	0.29					0.2	0.2	3	0.1	0.1	3	0.0	0.1	3	0.3	0.4
VI + IX	1.26	1.81	3	0.31	0.20	3	0.14	0.02	3	1.70	2.04					1.6	2.4	3	0.4	0.3	3	0.2	0.0	3	2.2	2.6
DEHP	13.39	6.92	3	0.49	0.21	3	0.04	0.01	3	13.92	7.14					17.4	9.0	3	0.6	0.3	3	0.1	0.0	3	18.1	9.3
Total	18.40	7.08	3	2.57	1.53	3	0.50	0.20	3	21.47	8.81					23.9	9.2	3	3.3	2.0	3	0.7	0.3	3	27.9	11.5

Excretion and metabolism study, repeated dosing

Table 12. Mean (and standard deviation) body weight values in control female mice and in female mice treated with a 9-day repeated oral administration (fromn D0 to D8 inclusive) of 200 or 1000 mg/kg/d [14 C]-DEHP.

Day	Control			200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
D-3	26.2	1.0	3	25.7	1.1	6	25.1	0.9	6
D0	24.0	0.7	3	24.9	1.3	6	23.4	0.8	6
D1	24.8	1.0	3	25.3	1.5	6	24.2	0.9	6
D2	25.4	0.8	3	25.4	2.0	6	23.7	1.0	6
D3	25.8	0.8	3	25.8	2.4	6	24.2	0.7	6
D4	26.3	1.0	3	26.1	2.2	6	24.2	1.0	5
D5	26.0	1.1	3	26.0	2.0	6	24.1	1.7	5
D6	26.5	1.2	3	26.2	2.4	6	24.1	2.4	5
D7	26.6	1.4	3	26.2	2.3	6	24.3	1.6	5
D8	26.9	1.5	3	26.6	2.7	6	25.0	1.5	5
D9	26.8	1.5	3	26.2	2.6	6	24.7	1.5	5
D10	26.7	1.4	3	25.9	2.7	6	24.5	1.6	5
D11	26.5	1.4	3	25.9	2.7	6	24.7	1.6	5

Table 13. Mean recovery of radioactivity in urine from female mice collected within 24 hours following the 1st to the 9th oral administration of 200 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 15.4 μ moles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

	Days	Daily excretion in μ mole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in μ mole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	4.8	3.1	2	31.2	20.1	2	4.8	3.1	2	31.2	20.1	2
R	2	6.4	0.6	2	41.3	3.7	2	11.2	2.5	2	36.3	8.2	2
E	3	6.9	2.0	2	45.0	13.2	2	18.1	0.5	2	39.2	1.1	2
A	4	4.3	0.9	2	28.0	5.6	2	22.4	1.4	2	36.4	2.2	2
T	5	5.7	4.0	2	36.9	25.7	2	28.1	2.6	2	36.5	3.4	2
M	6	7.1	1.2	2	46.3	8.1	2	35.2	3.8	2	38.1	4.2	2
E	7	No urine	-	2	-	-	2	35.2	3.8	2	32.7	3.6	2
N	8	5.8	1.5	2	37.8	9.9	2	41.0	5.4	2	33.3	4.3	2
I	9	6.0	0.6	2	38.8	3.7	2	47.0	5.9	2	33.9	4.3	2
O	10	1.9	0.7	2	-	-	-	48.9	6.6	2	35.3	4.8	2
D	11	1.2	0.6	2	-	-	-	50.1	7.3	2	36.2	5.2	2
RE													
CO													
VE													
RY													

Table 14. Mean recovery of radioactivity in faeces from female mice collected within 24 hours following the 1st to the 9th oral administration of 200 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 15.4 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	2.6	1.9	2	17.1	12.2	2	2.6	1.9	2	17.1	12.2	2
R	2	5.0	0.2	2	32.3	1.2	2	7.6	1.7	2	24.7	5.5	2
E	3	5.6	0.4	2	36.3	2.3	2	13.2	1.3	2	28.6	2.9	2
A	4	5.2	2.1	2	33.8	13.5	2	18.4	3.4	2	29.9	5.5	2
T	5	6.9	1.3	2	44.9	8.7	2	25.3	4.8	2	32.9	6.2	2
M	6	4.7	1.9	2	30.7	12.3	2	30.1	6.7	2	32.5	7.2	2
E	7	4.8	1.5	2	30.9	10.0	2	34.8	8.2	2	32.3	7.6	2
N	8	4.8	0.3	2	31.2	1.7	2	39.6	8.5	2	32.2	6.9	2
T	9	3.8	0.7	2	25.0	4.7	2	43.5	9.2	2	31.4	6.6	2
P	10	0.5	0.3	2	-	-	-	44.0	9.4	2	31.7	6.8	2
E	11	0.3	0.3	2	-	-	-	44.3	9.7	2	32.0	7.0	2
R													
I													
O													
D													
R													
E													
C													
O													
V													
E													
R													

Table 15. Mean recovery of radioactivity in urine and faeces from female mice collected within 24 hours following the 1st to the 9th oral administration of 200 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 15.4 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	7.4	5.0	2	48.4	32.3	2	7.4	5.0	2	48.4	32.3	2
R	2	11.3	0.8	2	73.6	5.0	2	18.8	4.2	2	61.0	13.7	2
E	3	12.5	2.4	2	81.3	15.5	2	31.3	1.8	2	67.7	4.0	2
A	4	9.5	2.9	2	61.8	19.1	2	40.8	4.8	2	66.3	7.8	2
T	5	12.6	2.6	2	81.8	17.0	2	53.4	2.2	2	69.4	2.8	2
M	6	11.9	0.7	2	77.0	4.3	2	65.3	2.8	2	70.7	3.0	2
E	7	4.8	1.5	2	30.9	10.0	2	70.1	4.4	2	65.0	4.0	2
N	8	10.6	1.3	2	69.0	8.2	2	80.7	3.1	2	65.5	2.5	2
T	9	9.8	0.2	2	63.8	1.0	2	90.5	3.3	2	65.3	2.3	2
P	10	2.4	0.4	2	-	-	-	92.9	2.8	2	67.0	2.0	2
E	11	1.5	0.3	2	-	-	-	94.4	2.5	2	68.1	1.8	2
R													
I													
O													
D													
R													
E													
C													
O													
V													
E													
R													
Y													

Table 16. Mean recovery of radioactivity in urine from female mice collected within 24 hours following the 1st to the 9th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 76.9 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	9.2	2.2	2	12.0	2.9	2	9.2	2.2	2	12.0	2.9	2
R	2	37.3	10.4	2	48.5	13.5	2	46.5	12.6	2	30.2	8.2	2
E	3	29.9	4.8	2	38.8	6.3	2	76.4	17.4	2	33.1	7.6	2
A	4	25.5	8.7	2	33.2	11.4	2	101.9	8.7	2	33.1	2.8	2
T	5	30.6	8.2	2	39.7	10.6	2	132.5	0.5	2	34.5	0.1	2
M	6	28.0	7.8	2	36.4	10.2	2	160.4	8.4	2	34.8	1.8	2
E	7	23.7	0.9	2	30.8	1.2	2	184.1	7.4	2	34.2	1.4	2
N	8	27.1	3.6	2	35.3	4.7	2	211.2	3.8	2	34.3	0.6	2
T	9	27.0	1.8	2	35.2	2.4	2	238.3	5.6	2	34.4	0.8	2
P	10	10.5	1.6	2	-	-	-	248.8	7.2	2	35.9	1.0	2
E	11	4.9	2.3	2	-	-	-	253.7	9.5	2	36.7	1.4	2
R													
I													
O													
D													
R													
E													
C													
O													
V													
E													
R													

Table 17. Mean recovery of radioactivity in faeces from female mice collected within 24 hours following the 1st to the 9th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 76.9 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T R E A T M E N T P E R I O D	1	6.3	3.6	2	8.2	4.7	2	6.3	3.6	2	8.2	4.7	2
	2	34.9	6.6	2	45.4	8.6	2	41.2	3.0	2	26.8	2.0	2
	3	32.1	6.6	2	41.8	8.6	2	73.3	9.6	2	31.8	4.2	2
	4	26.1	10.0	2	34.0	13.0	2	99.5	19.6	2	32.3	6.4	2
	5	28.7	5.7	2	37.3	7.5	2	128.2	13.9	2	33.3	3.6	2
	6	31.7	2.1	2	41.3	2.7	2	159.9	11.8	2	34.7	2.6	2
	7	25.0	2.5	2	32.5	3.2	2	184.9	9.3	2	34.4	1.7	2
	8	19.9	2.0	2	25.9	2.6	2	204.8	11.4	2	33.3	1.8	2
	9	19.4	3.6	2	25.2	4.7	2	224.2	15.0	2	32.4	2.2	2
R E C O V E R Y	10	1.6	0.2	2	-	-	-	225.8	15.2	2	32.6	2.2	2
	11	0.8	0.3	2	-	-	-	226.6	15.5	2	32.7	2.2	2

Table 18. Mean recovery of radioactivity in urine and faeces from female mice collected within 24 hours following the 1st to the 9th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Mice received a 9-day repeated oral administration of 76.9 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 584909 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	15.5	5.8	2	20.1	7.6	2	15.5	5.8	2	20.1	7.6	2
R	2	72.2	3.7	2	93.9	4.9	2	87.7	9.6	2	57.0	6.2	2
E	3	62.0	1.8	2	80.6	2.3	2	149.7	7.8	2	64.9	3.4	2
A	4	51.6	18.7	2	67.2	24.4	2	201.4	10.9	2	65.5	3.6	2
T	5	59.3	2.4	2	77.1	3.2	2	260.6	13.4	2	67.8	3.5	2
M	6	59.7	9.9	2	77.6	12.9	2	320.3	3.5	2	69.4	0.7	2
E	7	48.7	1.5	2	63.3	2.0	2	369.0	1.9	2	68.6	0.4	2
N	8	47.0	5.6	2	61.1	7.3	2	416.0	7.5	2	67.6	1.2	2
T	9	46.4	1.8	2	60.4	2.3	2	462.5	9.3	2	66.8	1.3	2
P	10	12.1	1.4	2	-	-	-	474.6	8.0	2	68.6	1.2	2
E	11	5.7	2.0	2	-	-	-	480.3	6.0	2	69.4	0.9	2
R													
I													
O													
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RE													
CO													
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RY													

Table 19. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in µmoles) in urine from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 200 mg/kg/d.

	Treatment	1 st			4 th			7 th		
	Metabolites	Mean	SD	n	Mean	SD	n	Mean	SD	n
F R E E	MEHP	1.10	0.09	2	1.20	0.33	2	No urine		2
	I	0.35	0.28	2	0.38	0.13	2		2	
	II	0.11	0.08	2	0.15	0.13	2		2	
	IV	0.16	0.16	2	0.13	0.10	2		2	
	V	0.17	0.17	2	0.21	0.11	2		2	
	VI	0.59	0.48	2	0.39	0.09	2		2	
	VII	0.14	0.07	2	0.15	0.06	2		2	
	IX	0.91	0.89	2	0.45	0.11	2		2	
	XVII	0.27	0.09	2	0.24	0.04	2		2	
	XXVI	0.20	0.07	2	0.17	0.03	2		2	
	DEHP	0.05	0.04	2	0.07	0.04	2		2	
	Total	4.05	2.42	2	3.53	0.51	2		2	
C O N J U G A T E	MEHP	0.09	0.10	2	0.01	0.02	2	No urine		2
	I	0.16	0.13	2	0.19	0.01	2		2	
	II	0.05	0.07	2	0.00	0.00	2		2	
	IV	0.01	0.00	2	0.00	0.00	2		2	
	V	0.03	0.03	2	0.00	0.00	2		2	
	VI	0.10	0.10	2	0.17	0.08	2		2	
	VII	0.03	0.04	2	0.00	0.00	2		2	
	IX	0.27	0.22	2	0.36	0.22	2		2	
	XVII	0.00	0.00	2	0.02	0.02	2		2	
	XXVI	0.01	0.01	2	0.01	0.01	2		2	
	Total	0.75	0.69	2	0.77	0.34	2		2	

Table 19 (continued)

	Treatment	1 st			4 th			7 th			
	Metabolites	Mean	SD	n	Mean	SD	n	Mean	SD	n	
T O T A L	MEHP	1.19	0.19	2	1.21	0.31	2	No urine			2
	I	0.51	0.41	2	0.57	0.11	2				2
	II	0.16	0.16	2	0.15	0.13	2				2
	IV	0.17	0.15	2	0.13	0.10	2				2
	V	0.20	0.20	2	0.21	0.11	2				2
	VI	0.69	0.57	2	0.56	0.17	2				2
	VII	0.17	0.12	2	0.15	0.07	2				2
	IX	1.18	1.11	2	0.81	0.33	2				2
	XVII	0.27	0.09	2	0.26	0.06	2				2
	XXVI	0.21	0.08	2	0.18	0.04	2				2
	DEHP	0.05	0.04	2	0.07	0.04	2				2
	Total	4.80	3.11	2	4.30	0.85	2				2

Table 20. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 200 mg/kg/d.

Treatment	1 st			4 th			7 th		
Metabolites	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	0.48	0.49	2	1.07	0.06	2	0.49	0.10	2
I	0.05	0.03	2	0.12	0.04	2	0.05	0.01	2
IV	0.00	0.00	2	0.04	0.02	2	0.01	0.00	2
V	0.08	0.07	2	0.18	0.03	2	0.09	0.01	2
VII	0.01	0.01	2	0.07	0.03	2	0.02	0.00	2
VI + IX	0.11	0.11	2	0.28	0.16	2	0.09	0.00	2
DEHP	1.93	1.22	2	3.43	1.98	2	4.05	1.64	2
Total	2.65	1.91	2	5.20	2.12	2	4.80	1.56	2

Table 21. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg/d.

Treatment		1 st			4 th			7 th		
Metabolites		Mean	SD	n	Mean	SD	n	Mean	SD	n
F R E E	MEHP	2.62	0.33	2	10.40	5.39	2	11.17	2.37	2
	I	0.80	0.32	2	3.60	1.02	2	2.89	0.43	2
	II	0.12	0.00	2	0.23	0.01	2	0.19	0.04	2
	IV	0.15	0.02	2	0.61	0.17	2	0.26	0.02	2
	V	0.24	0.17	2	0.85	0.06	2	0.56	0.15	2
	VI	0.90	0.26	2	3.05	0.98	2	2.57	0.20	2
	VII	0.18	0.04	2	0.55	0.24	2	0.33	0.05	2
	IX	1.91	0.91	2	3.20	0.56	2	2.52	0.31	2
	XVII	0.23	0.04	2	0.63	0.01	2	0.44	0.06	2
	XXVI	0.15	0.06	2	0.49	0.31	2	0.51	0.38	2
	DEHP	0.04	0.01	2	0.11	0.06	2	0.13	0.01	2
	Total		7.34	2.10	2	23.72	7.34	2	21.58	1.13
C O N J U G A T E	MEHP	0.01	0.01	2	0.34	0.39	2	0.52	0.16	2
	I	0.33	0.07	2	0.07	0.04	2	0.22	0.31	2
	II	0.00	0.00	2	0.01	0.01	2	0.02	0.01	2
	IV	0.02	0.02	2	0.00	0.00	2	0.00	0.00	2
	V	0.07	0.07	2	0.03	0.04	2	0.02	0.03	2
	VI	0.55	0.11	2	0.47	0.54	2	0.61	0.23	2
	VII	0.00	0.00	2	0.00	0.00	2	0.00	0.00	2
	IX	0.88	0.06	2	0.72	0.33	2	0.65	0.38	2
	XVII	0.00	0.00	2	0.13	0.19	2	0.01	0.02	2
	XXVI	0.00	0.01	2	0.00	0.01	2	0.00	0.01	2
Total		1.86	0.17	2	1.78	1.42	2	2.07	0.22	2

Table 21 (continued)

Treatment	1 st			4 th			7 th		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	2.63	0.35	2	10.74	5.78	2	11.69	2.53	2
I	1.13	0.39	2	3.67	1.06	2	3.12	0.12	2
II	0.12	0.00	2	0.24	0.02	2	0.22	0.05	2
IV	0.16	0.00	2	0.60	0.17	2	0.26	0.03	2
V	0.31	0.10	2	0.88	0.01	2	0.59	0.18	2
VI	1.44	0.37	2	3.52	1.51	2	3.19	0.03	2
VII	0.18	0.03	2	0.56	0.24	2	0.33	0.06	2
IX	2.80	0.97	2	3.93	0.89	2	3.17	0.69	2
XVII	0.23	0.04	2	0.76	0.18	2	0.46	0.08	2
XXVI	0.15	0.07	2	0.49	0.32	2	0.51	0.38	2
DEHP	0.04	0.01	2	0.11	0.05	2	0.13	0.01	2
Total	9.20	2.26	2	25.50	8.77	2	23.65	0.92	2

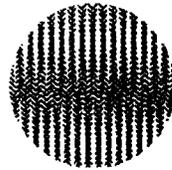
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Table 22. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female mice collected within 24 hours following the 1st, 4th and 7th oral administration of 1000 mg/kg/d.

Metabolites	1 st			4 th			7 th		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	0.53	0.53	2	0.95	0.32	2	0.97	0.15	2
I	0.04	0.04	2	0.13	0.03	2	0.11	0.04	2
IV	0.01	0.00	2	0.02	0.00	2	0.01	0.00	2
V	0.14	0.11	2	0.10	0.04	2	0.13	0.01	2
VII	0.02	0.02	2	0.04	0.00	2	0.03	0.01	2
VI + IX	0.07	0.07	2	0.24	0.03	2	0.14	0.03	2
DEHP	5.43	2.84	2	24.69	9.55	2	23.65	2.25	2
Total	6.25	3.61	2	26.15	9.97	2	25.05	2.47	2



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**DI-(2-ETHYLHEXYL)PHTHALATE (DEHP)
ABSORPTION, EXCRETION, METABOLISM AND
PHARMACOKINETIC PROFILE IN WISTAR FEMALE RATS**

REPORT no. 1/99

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ABSORPTION, EXCRETION, METABOLISM AND
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I have reviewed this report and concur with its content.

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Date 
22.12.2000

I, the undersigned, was responsible for the conduct of the work and reporting of the results. I concur with the views expressed in the report.

Laurence Laignelet, PhD

(Study director)

Date 
22.12.2000

CONTENTS

Title page	
Signature page	1
1. SUMMARY	7
2. INTRODUCTION	9
3. MATERIALS	11
3.1 Test Material	11
3.1.1 Non-radioactive test material.....	11
3.1.2 Radiolabelled test material.....	11
3.2 Reagents and chemical	11
3.3 Animals	11
3.4 Instrumentation	11
4. METHOD	12
4.1 Animals	12
4.1.1 Identification	12
4.1.2 Acclimatisation.....	12
4.1.3 Environmental control.....	12
4.1.4 Animal accommodation.....	12
4.1.5 Cage identification.....	12
4.1.6 Diet and water supply.....	12
4.2 Determination of radioactivity.....	12
4.2.1 Background radioactivity	13
4.2.2 Limit of detection	13
4.3 Gas chromatography analysis	13
4.3.1 Gas chromatography conditions.....	13
4.3.2 Quantification.....	13
4.3.3 MEHP-derived metabolites identification	13
4.4 Dose formulation.....	15
4.5 Administration	15
4.6 Treatment groups.....	15
4.6.1 Blood radioactivity	15
4.6.1.1 Single oral dose.....	15
4.6.1.2 Repeated oral dose	16
4.6.2 Mass balance excretion study.....	16
4.6.2.1 Single oral dose	16
4.6.2.2 Repeated oral dose	16
4.7 Body weights.....	16
4.8 Sampling and storage.....	16
4.8.1 Identification	16
4.8.2 Urine and faeces	16
4.8.3 Blood and plasma.....	17
4.9 Sample analysis.....	17
4.9.1 Radioactivity analysis	17
4.9.1.1 Dose solution	17
4.9.1.2 Urine	17
4.9.1.3 Faeces	17
4.9.1.4 Blood.....	18
4.9.2 Gas chromatography analysis.....	18
4.9.2.1 Urine	18
4.9.2.2 Faeces	18
4.9.2.3 Preparation of derivatives.....	18
4.10 Pharmacokinetic data analysis	18

5. RESULTS	19
5.1 Pharmacokinetic study	19
5.1.1 Single dosing	19
5.1.1.1 Body weights	19
5.1.1.2 Recovery of radioactivity	19
5.1.1.2.1 Dose level 200 mg/kg	19
5.1.1.2.2 Dose level 1000 mg/kg	19
5.1.2 Repeated dosing	19
5.1.2.1 Body weights	19
5.1.2.2 Recovery of radioactivity	19
5.1.2.2.1 Dose level 200 mg/kg	19
5.1.2.2.2 Dose level 1000 mg/kg	20
5.2 Excretion and metabolism study	20
5.2.1 Single dosing	20
5.2.1.1 Body weights	20
5.2.1.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces	20
5.2.1.2.1 Recovery of radioactivity	20
5.2.1.2.1.1 Dose level 200 mg/kg	20
5.2.1.2.1.2 Dose level 1000 mg/kg	20
5.2.1.2.2 Analysis by gas chromatography	21
5.2.1.2.2.1 Dose level 200 mg/kg	21
5.2.1.2.2.2 Dose level 1000 mg/kg	21
5.2.2 Repeated dosing	22
5.2.2.1 Body weights	22
5.2.2.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces	22
5.2.2.2.1 Recovery of radioactivity	22
5.2.2.2.1.1 Dose level 200 mg/kg	22
5.2.2.2.1.2 Dose level 1000 mg/kg	23
5.2.2.2.2 Analysis by gas chromatography	24
5.2.2.2.2.1 Dose level 200 mg/kg	24
5.2.2.2.2.2 Dose level 1000 mg/kg	25
6. DISCUSSION	28
7. REFERENCES	30
FIGURES	31
Pharmacokinetic study	31
<u>Figure 1.</u> Concentration of radioactivity in blood (in nmole equivalent of DEHP per gram of blood) from female rats following a single oral administration of [¹⁴ C]-DEHP at 200 or 1000 mg/kg.	31
<u>Figure 2.</u> Concentration of radioactivity in blood (in nmole equivalent of DEHP per gram of blood) from female rats following a single oral administration of 200 or 1000 mg/kg [¹⁴ C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled-DEHP, respectively.	32
Excretion and metabolism study, single dosing	33
<u>Figure 3.</u> Recovery of radioactivity (in % of the DEHP dose) in urine and faeces from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg [¹⁴ C]-DEHP.	33
<u>Figure 4.</u> Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	34
<u>Figure 5.</u> Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	35
<u>Figure 6.</u> Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	36
<u>Figure 7.</u> Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.	37
<u>Figure 8.</u> Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in the urine and faeces of female rats treated with a single oral dose of 200 mg/kg or 1000 mg/kg DEHP	38

Excretion and metabolism study, repeated dosing.....	39
Figure 9. Mean recovery of radioactivity (in μ mole DEHP-equivalents) in urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 200 mg/kg/d [¹⁴ C]-DEHP.....	39
Figure 10. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 200 mg/kg/d [¹⁴ C]-DEHP.....	40
Figure 11. Mean recovery of radioactivity (in μ mole DEHP-equivalents) in urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP.....	41
Figure 12. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP.....	42
Figure 13. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female rats within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.....	43
Figure 14. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female rats within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.....	44
Figure 15. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in the urine and faeces of female rats within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 200 mg/kg [¹⁴ C]-DEHP.....	45
Figure 16. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female rats within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.....	46
Figure 17. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female rats within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.....	47
Figure 18. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in the urine and faeces of female rats within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 1000 mg/kg [¹⁴ C]-DEHP.....	48
TABLES.....	49
Pharmacokinetic study.....	49
Table 1. Mean (and standard deviation) body weight values in female rats treated with a single administration of 200 or 1000 mg/kg DEHP on Day 0.....	49
Table 2. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g of blood) from female rats following a single oral administration of 200 or 1000 mg/kg [¹⁴ C]-DEHP.....	49
Table 3. Mean (and standard deviation) body weight values in female rats treated with a 6-day repeated oral administration of 200 or 1000 mg/kg DEHP from D0 to D5 (inclusive).....	50
Table 4. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g of blood) from female rats after a single administration 200 or 1000 mg/kg of [¹⁴ C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled DEHP, respectively.....	51
Excretion and metabolism study, single dosing.....	52
Table 5. Mean (and standard deviation) body weight values in control female rats and in female rats treated with a single administration of 200 or 1000 mg/kg [¹⁴ C]-DEHP on Day 0.....	52
Table 6. Recovery of radioactivity (as percentage of the [¹⁴ C]-DEHP dose and as μ mole DEHP-equivalents) in urine and faeces from female rats following a single oral administration of 200 mg/kg.....	53
Table 7. Recovery of radioactivity (in % of the [¹⁴ C]-DEHP dose and μ mole DEHP-equivalents) in urine and faeces from female rats following a single oral administration of 1000 mg/kg.....	53
Table 8. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in urine from female rats following a single oral administration of 200 mg/kg.....	54
Table 9. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in faeces from female rats following a single oral administration of 200 mg/kg.....	55
Table 10. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in urine from female rats following a single oral administration of 1000 mg/kg.....	56
Table 11. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in faeces from female rats following a single oral administration of 1000 mg/kg.....	57

Excretion and metabolism study, repeated dosing	58
<u>Table 12.</u> Mean (and standard deviation) body weight values in control female rats and in female rats treated with a 10-day repeated oral administration of 200 or 1000 mg/kg [¹⁴ C]-DEHP	58
<u>Table 13.</u> Mean recovery of radioactivity in urine from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 200 mg/kg/d [¹⁴ C]-DEHP	59
<u>Table 14.</u> Mean recovery of radioactivity in faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 200 mg/kg/d [¹⁴ C]-DEHP	60
<u>Table 15.</u> Mean recovery of radioactivity in urine from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP	61
<u>Table 16.</u> Mean recovery of radioactivity in faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administration of 1000 mg/kg/d [¹⁴ C]-DEHP	62
<u>Table 17.</u> Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in µmoles) in urine from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 200 mg/kg/d	63
<u>Table 18.</u> Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in µmoles) in faeces from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 200 mg/kg/d	64
<u>Table 19.</u> Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in µmoles) in urine from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 1000 mg/kg/d	65
<u>Table 20.</u> Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in µmoles) in faeces from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administration of 1000 mg/kg/d	66
APPENDIXES	67
Pharmacokinetic study	67
<u>Appendix 1.</u> Individual body weight values for female rats treated with a single oral administration of 200 mg/kg (rats 1 to 6) or 1000 mg/kg (rats 7 to 12) of [¹⁴ C]-DEHP on day 0	67
<u>Appendix 2.</u> Recovery of radioactivity in blood (in dpm/g blood and nmole DEHP-equivalents/g blood) from female rats following a single oral administration of [¹⁴ C]-DEHP	68
<u>Appendix 3.</u> Individual body weight values for female rats treated with a 6-day repeated oral administration of 200 mg/kg/d DEHP from D0 to D5 (inclusive)	69
<u>Appendix 4.</u> Individual body weight values for female rats treated with a 6-day repeated oral administration of 1000 mg/kg/d DEHP from D0 to D5 (inclusive)	70
<u>Appendix 5.</u> Recovery of radioactivity in blood (in dpm/g blood and nmole DEHP-equivalents/g blood) from female rats following a single oral administration of [¹⁴ C]-DEHP which was preceded by a 5-day treatment with unlabelled DEHP	71
Excretion and metabolism study, single dosing	72
<u>Appendix 6.</u> Individual body weight values for control female rats (T1 to T3) and female rats treated with a single oral dose of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6) [¹⁴ C]-DEHP	72
<u>Appendix 7.</u> Individual recovery of radioactivity (in dpm) in the urine and faeces from female rats following a single oral administration of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6)	73
<u>Appendix 8.</u> Individual calculated recovery of radioactivity (in % of the dose or µmole DEHP-equivalents) excreted in urine and faeces from female rats following a single oral administration of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6)	74
<u>Appendix 9.</u> Gas chromatography analysis of urine of female rats treated with a single oral dose of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6) DEHP. Peak areas values and percentage of total for each metabolites	75
<u>Appendix 10.</u> Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (in µmoles or % of the dose) in urine from female rats following a single oral administration of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6) DEHP	77
<u>Appendix 11.</u> Recovery of [¹⁴ C]-DEHP (in % of total radioactivity) in the faeces from female rats following a single administration of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6) [¹⁴ C]-DEHP	79
<u>Appendix 12.</u> Gas chromatography analysis of faeces from female rats following a single oral administration of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6) DEHP. Peak areas values and percentage of total for each metabolites	80
<u>Appendix 13.</u> Individual calculated amount of DEHP and MEHP-derived metabolites (as µmoles or as % of the dose) identified in faeces from female rats following a single oral administration of 200 mg/kg (rats 1 to 3) or 1000 mg/kg (rats 4 to 6) DEHP	81

Excretion and metabolism study, repeated dosing	82
<u>Appendix 14.</u> Individual body weight values for control female rat (T1) and female rats treated with a 10-day repeated oral administration of 200 mg/kg/d (rats 2 to 6) or 1000 mg/kg/d (rats 7 to 11) of [¹⁴ C]-DEHP from D0 to D9.	82
<u>Appendix 15.</u> Individual recovery of radioactivity (in dpm) in the urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administrations of 200 mg/kg/d.	83
<u>Appendix 16.</u> Individual calculated amount of radioactivity in the urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administrations of 200 mg/kg/d.	88
<u>Appendix 17.</u> Individual recovery of radioactivity (in dpm) in the urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administrations of 1000 mg/kg/d.	93
<u>Appendix 18.</u> Individual calculated amount of radioactivity in the urine and faeces from female rats collected within 24 hours following the 1 st to the 10 th oral administrations of 1000 mg/kg/d.	98
<u>Appendix 19.</u> Gas chromatography analysis of urine from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administrations of 200 mg/kg/d (rats 2 to 6) or 1000 mg/kg/d (rats 7 to 11) [¹⁴ C]-DEHP. Peak areas values and percentage of total for DEHP, MEHP and MEHP-derived metabolites.	103
<u>Appendix 20.</u> Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (in µmoles) present in urine from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administrations of 200 mg/kg/d (rats 2 to 6) or 1000 mg/kg/d (rats 7 to 11) of DEHP.	108
<u>Appendix 21.</u> Recovery of [¹⁴ C]-DEHP in the faeces from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administrations of 200 mg/kg/d [¹⁴ C]-DEHP.	113
<u>Appendix 22.</u> Recovery of [¹⁴ C]-DEHP in the faeces from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administrations of 1000 mg/kg/d [¹⁴ C]-DEHP.	114
<u>Appendix 23.</u> Gas chromatography analysis of faeces from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administrations of 200 mg/kg/d (rats 2 to 6) or 1000 mg/kg/d (rats 7 to 11) [¹⁴ C]-DEHP. Peak areas values and percentage of total for DEHP, MEHP and MEHP-derived metabolites.	115
<u>Appendix 24.</u> Individual calculated amount of DEHP, MEHP and MEHP-derived metabolites (in µmoles) present in faeces from female rats collected within 24 hours following the 1 st , 4 th , 7 th and 10 th oral administrations of 200 mg/kg/d (rats 2 to 6) or 1000 mg/kg/d (rats 7 to 11).	118

1. SUMMARY

Di(2-ethylhexyl)phthalate (DEHP) is extensively used as plasticiser for polyvinyl chloride. The absorption, blood concentration and excretion of DEHP was determined in Wistar female rats following a single and a repeated oral administration at the dose levels of 200 mg/kg and 1000 mg/kg. Blood samples were taken at defined time intervals after administration for quantification of total radioactivity. Urine and faeces were collected daily and DEHP and its metabolites were extracted and then identified by GC-MS and quantified by GC.

The absorption of radioactive material following single doses of [¹⁴C]-DEHP at 200 mg/kg and 1000 mg/kg to female Wistar rats was rapid. The maximum blood concentrations (C_{max}), approximately 64 and 353 nmole DEHP-equivalents/g for the low and the high doses, were obtained 1.5 and 4 hours after dosing, respectively. The half-life of elimination were approximately 7.1 and 10.2 hours at the low and the high dose level, respectively. The areas under the curves (AUC_{0-48h}) were 1426 and 5825 nmole DEHP-equivalents.h.g⁻¹ for 200 mg/kg and 1000 mg/kg dose levels, respectively. This 4.1-fold difference in AUC is consistent with the blood profile in which there is a 5.5-fold difference in the maximum concentration between dose levels and the 5-fold difference in dose levels. After 48 hours, no radioactivity was detected in blood samples following 200 mg/kg dose, and a level representing 5% of the C_{max} was still present following 1000 mg/kg dose.

After a 5-day pre-treatment of female rats with unlabelled DEHP at 200 or 1000 mg/kg bw/d, the absorption of radioactive material following a single dose of [¹⁴C]-DEHP at 200 mg/kg and 1000 mg/kg was rapid. The maximum blood concentrations (C_{max}), approximately 77 and 405 nmole DEHP-equivalents/g for the low and the high doses, were obtained 1.5 hours after dosing, respectively. The half-life of elimination were approximately 8.7 and 13.7 hours at the low and the high dose level, respectively. The AUC_{0-48h} were 1007 and 6398 nmole DEHP-equivalents.h.g⁻¹ for 200 mg/kg and 1000 mg/kg dose levels respectively. This 6.3-fold difference in AUC is still consistent with the blood profile in which there is a 5.2-fold difference in the maximum concentration between dose levels and the 5-fold difference in dose levels. After 48 hours, a very low level of radioactivity was detected in blood samples following the 200 mg/kg dose, and a level representing 10% of the C_{max} was still present following the 1000 mg/kg dose.

According to the previous results, and whatever the dose level, a 5-day pre-treatment with unlabelled DEHP did not affect significantly the AUC_{0-48h} of a further [¹⁴C]-DEHP dose. However, the half-life of elimination at the low and high dose level increased from 7.1 and 10.2 hours after a single administration to 8.7 and 13.5 hours after a repeated administration, respectively.

After a single oral administration, excretion of radioactive material was very rapid as most was excreted in the first 48 hours. Following the low and high dose administrations, within 96 hours, approximately 58% and 47% of the dose were recovered in the urine and approximately 31% and 41% in the faeces, respectively. The total overall mean recoveries were approximately 89% at both dose levels. Approximately 17% and 30% of the low and high DEHP dose were excreted unchanged almost totally in faeces, either due to a non absorption or/and a re-excretion as a consequence of an entero-hepatic re-circulation. MEHP, the proximate metabolite issued from DEHP hydrolysis, was also almost totally excreted in faeces (12% and 7% of the DEHP dose at 200 mg/kg and 1000 mg/kg, respectively). MEHP-derived metabolites were mainly excreted in urine (53% and 45% of the DEHP dose at 200 and 1000 mg/kg, respectively) and a small part in faeces (8% and 5% of the dose at 200 mg/kg and 1000 mg/kg, respectively). Total excretion of MEHP-derived metabolites reached 61 and 50% of the dose at 200 mg/kg and 1000 mg/kg, respectively. According to the blood concentrations observed, such difference could be related to a saturation of the oxidation pathways rather than a saturation of DEHP absorption or/and DEHP hydrolysis. Whatever the dose level, MEHP ω -1 oxidation was the main metabolic pathway (about 60%), followed by ω -oxidation (about 40%).

During a 10-day repeated administration of [¹⁴C]-DEHP, the urine and faecal excretion reached a steady-state after 4 days. Approximately 69% and 73% of the total [¹⁴C]-DEHP dose were recovered in the urine and 23% and 28% in the faeces at 200 and 1000 mg/kg/d, respectively. At 200 mg/kg/d, the excretion in urine decreased slightly after the 7th administration and increased in faeces from the 1st administration. No such effect was observed at 1000 mg/kg/d. The total overall mean recovery of radioactive material in excreta was similar to that obtained following a single administration at 200 mg/kg/d, approximately 92% and slightly higher at 1000 mg/kg/d, approximately 101%. These values

of total excretion and the observed steady-state excluded any significant bio-accumulation of DEHP.

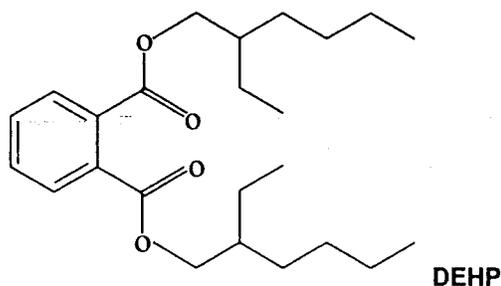
During the repeated administration of 200 mg/kg bw/d DEHP for 10 days to female rats, the excretion of non-metabolised DEHP decreased significantly and represented 14, 5, 6 and 1% of the total excretion within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. DEHP was essentially excreted in faeces after the 1st administration (94%), then the part of the faecal excretion of DEHP represented only 64% after the 4th and 7th administration and 58% after the 10th administration, respectively. The excretion of MEHP was stable and represented respectively 12, 7, 11 and 10% of the total excretion within 24 hours after the 1st, 4th, 7th and 10th administration. Eighty-two to 93% of MEHP were excreted in faeces. The excretion of MEHP-derived metabolites increased slightly with time and represented 75, 87, 83 and 89% of the total excretion after the 1st, 4th, 7th and 10th administration, respectively. Ninety percent after the 1st and the 4th administrations and 80% after the 7th and 10th administrations of these metabolites were recovered in urine. The total excretion of metabolites issued from ω -1 oxidation (VI, IX, XVII and XXVI) or ω -oxidation (I, II, IV, V and VII) represented 62, 60, 67 and 63% or 38, 40, 33, 37% of the MEHP-derived metabolites excreted after the 1st, 4th, 7th and 10th administrations, respectively.

During the repeated administration of 1000 mg/kg bw/d DEHP for 10 days to female rats, the excretion of non-metabolised DEHP decreased significantly from 33 and 19% of the total excretion after the 1st and 4th administration to 6 and 8% after the 7th and 10th administration, respectively. DEHP was almost totally excreted in faeces after the 1st administration (>99%), then the part of the faecal excretion of DEHP represented about 94% after the 4th administration. Within 24 hours after the 1st, 4th, 7th and 10th administration, the excretion of MEHP was stable and represented 5, 10, 9 and 9% of the total excretion and 91, 69, 82 and 89% of MEHP were excreted in faeces, respectively. The part of MEHP-derived metabolites in the total excretion increased slightly with time and represented 61, 71, 84 and 84% after the 1st, 4th, 7th and 10th administrations, respectively. Ninety-nine, 95, 88 and 90% percent of these metabolites were excreted in urine after the 1st, 4th, 7th and 10th administration. Metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII), represented 70, 66, 72 and 72% and 30, 34, 28 and 28% of the total of the MEHP-derived metabolites excreted within 24 hours after the 1st, 4th, 7th and 10th administrations, respectively.

In conclusion, [¹⁴C]-DEHP was rapidly, extensively and dose-related absorbed following a single or repeated oral administration to female Wistar rats as mirrored by the blood concentration curve profile and a rapid excretion in urine and faeces. A 5-day pre-treatment did not have any significant effect on the total absorption rate but increased slightly the half-life of elimination at the high dose level. After a single or a repeated administration, [¹⁴C]-DEHP was excreted very quickly in excreta and the recovery reached or exceeded 90% of the administered dose. [¹⁴C]-DEHP was excreted in majority as MEHP-derived metabolite essentially in urine, as MEHP mainly in faeces and DEHP almost totally in faeces. Omega-1 oxidation was the main metabolic pathway (c.a. 60-70%) of the production of MEHP-derived metabolite. The repeated administration was characterised by a decrease of the DEHP excretion and a concomitant increase of the MEHP-derived metabolites but without alteration of the ω/ω -1 oxidation ratio. This effect was probably related to the metabolic activation which took place after a few days of treatment.

2. INTRODUCTION

Di-(2-ethylhexyl) phthalate (DEHP) is a phthalate ester which is also known as 1,2-benzenedicarboxylic acid di-(2-ethylhexyl) ester. It is extensively used in industry as a plasticiser for flexible polyvinyl chloride products.

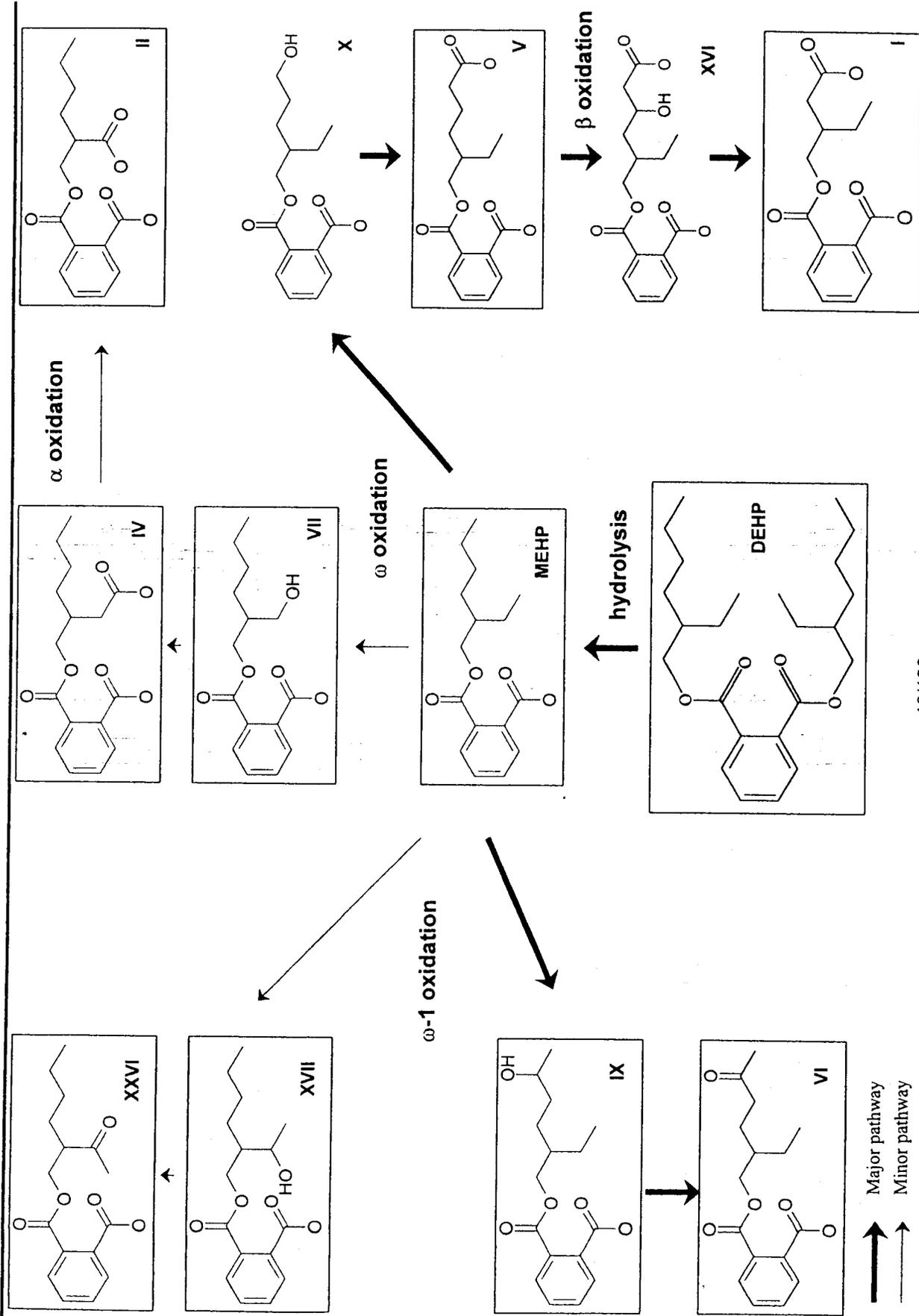


This report describes the absorption, excretion, metabolism and pharmacokinetics of DEHP in female rats after a single and repeated administration.

The structural relationships of the MEHP metabolites are summarised in the following scheme adapted from Lhuguenot et al. (1985). Only the framed metabolites have been identified in this study.

This study was performed at ENSBANA between September 1997 and April 1999.

A copy of the final report and all the primary data pertaining to the study have been retained in the Food Toxicology Laboratory of ENSBANA.



3 MATERIALS

3.1 Test Material

3.1.1 Non-radioactive test material

Unlabelled di(2-ethylhexyl)phthalate (DEHP, CAS Reg. no. 117-81-7, chemical purity > 99%) was obtained from Imperial Chemical Industries (Baleycourt, France). It was a clear, colourless liquid which arrived at ENSBANA on September 1997 and was stored, at 2-8°C, in the dark.

3.1.2 Radiolabelled test material

DEHP uniformly labelled on the phenyl ring ($[^{14}\text{C}]$ -DEHP, CAS Reg. no. 82208-43-3, batch 027H9218, 5.1 mCi/mmol), was purchased from Sigma (La Verpillère, France). Chemical and radiochemical purity was determined by HPLC and shown to be more than 98%.

3.2 Reagents and chemical

Mono(4-methylhexyl)phthalate (4-MHP), used as internal standard in the assays of plasma and urinary DEHP-derived metabolites, was synthesised in ENSBANA laboratory with a chemical purity greater than 95%.

Dibutyl phthalate (DBP, CAS Reg. No. 84-74-2), used as internal standard in the fecal DEHP-derived metabolites analysis, was obtained from Sigma.

Scintillation solutions, Hisafe II and Hionic Fluor, were obtained from Berthold and Wallac (France) and Packard Instrument Co. (Rungis, France), respectively. Soluene-350 was obtained from Packard Instrument Co. (Rungis, France). All the others chemicals were of the highest available purity and were purchased from Prolabo (Dijon, France).

3.3 Animals

SPF female Wistar rats were purchased from Iffa-Credo (Lyon, France). Rats were ten to twelve weeks old at the start of the experiments and their weights ranged between 200-220g.

3.4 Instrumentation

The carbon-14 was determined using an scintillation spectrometer (Packard Tri-Carb instrument, Model 2100 TR) calibrated for carbon-14 measurement using a quenched carbon-14 series and external standard spectral quench parametric analysis.

Solid sample were homogenised using an Ika-Werk homogeniser (Roucaire, Courtaboeuf, France).

DEHP-metabolites previously identified by GC-MS were quantified by GC. All chromatographic runs were performed on Chrompack CP 9000 equipped with a flame ionisation detector. Data analysis was performed by a HP Model 604 integrator. Capillary column was a Chrompack OV-1701 (stationary phase: 86% diméthyl silicon, 7% Phenyl silicon, 7% Cyanopropyl silicon).

4. METHOD

4.1 Animals

4.1.1 Identification

Each rat was assigned a number and identified within the study by a tailmark.

4.1.2 Acclimatisation

The rats were allowed to acclimatise to the laboratory conditions for a least three days before commencement of treatment.

4.1.3 Environmental control

The rats were housed in an environment of $23\pm 2^{\circ}\text{C}$ with a 12h dark/light cycle.

4.1.4 Animal accommodation

Animals were housed individually in metabolism cage (Nalgène) with wire mesh floors, equipped for the separate collection of urine and faeces.

Cage, cage trays, food hoppers and water bottles were changed at appropriate intervals.

4.1.5 Cage identification

Labels identifying the rat by experiment, animal number and treatment group were placed on each cage.

4.1.6 Diet and water supply

Rats had free access to food (AO₄ pellet diet, U.A.R., Epinay sur Orge, France) and tap water.

4.2 Determination of radioactivity

The radioactivity in all samples was determined by liquid scintillation spectrometry (counting) either by direct addition of the sample to the scintillation solution (Hisafe II, Berthold and Wallac, France) or after pre-treatment.

All samples were counted until a 2σ -value equal to 1.5% is reached, or if not the maximum counting time was 5 minutes.

4.2.1 Background radioactivity

The background radioactivity was determined for each series of analyses. For liquid samples (urine and blood), the background radioactivity was determined using the addition of an equivalent volume of water to the scintillation fluid in a vial. The first vial was counted for ten minutes to determine the background radioactivity. This background was automatically subtracted from the samples counts of the considered series.

4.2.2 Limit of detection

The limit of detection (LOD) was derived statistically from the background counts so that there was 98.5% certainty that samples with a mean value greater than the limit of detection contained radioactivity from [¹⁴C]-DEHP.

The limit of detection throughout the study was approximately 3 dpm, this is equivalent to about 0.3 nmole DEHP-equivalents for the low dose and 1.5 nmole DEHP-equivalents for the high dose.

4.3 Gas chromatography analysis

Quantitative determination of the metabolite profile was performed by gas chromatography (GC).

4.3.1 Gas chromatography conditions

A 25m x 0.32mm and a 50m x 0.32mm columns were used for urine/plasma and faeces chromatographic runs, respectively. The separations were carried out with an oven temperature programmed from 150°C to 260°C at 4°C/min. Nitrogen (0.8 bar) was the carrier gas. Injector and detector port temperatures were 250°C and 280°C, respectively.

4.3.2 Quantification

Quantification was based on peak areas of the flame ionisation detector corrected as relative molar detector response when a reference compound was available, otherwise a relative molar detector response was estimated. Total amounts of parent compound and/or metabolites were calculated from radioactivity present in the urine, faeces and blood extract. In urine, blood and faeces, GC profile data was used to estimate the sum of metabolites, reaching nearly 100% of the previous amount.

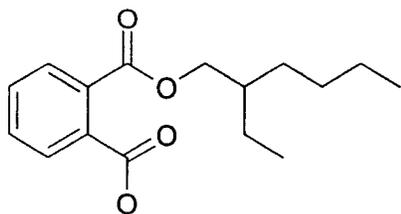
4.3.3 MEHP-derived metabolites identification

MEHP-derived metabolites were identified by gas chromatography-mass spectrometry. The detailed procedure and the results are reported in an independent report (*ENSBANA* addendum to reports no. 1/99 to 4/99).

The numbering proposed by Albro and Lavenhar (1989) was adopted in this study.

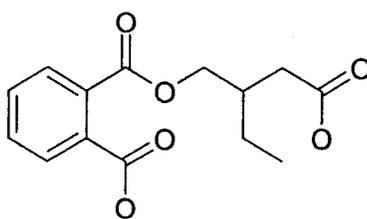
MEHP

MW: 278



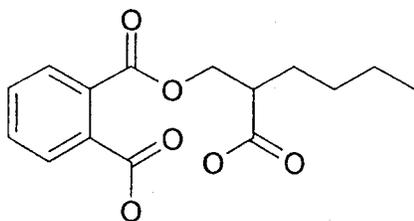
Metabolite I

MW: 280



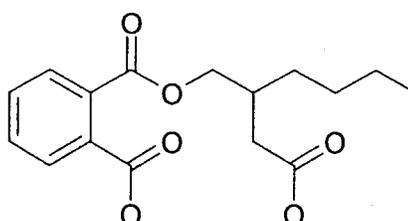
Metabolite II

MW: 294



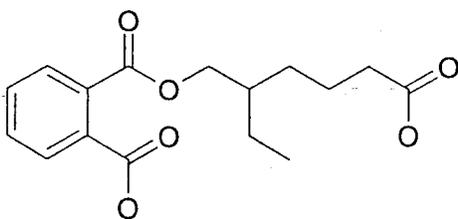
Metabolite IV

MW: 308



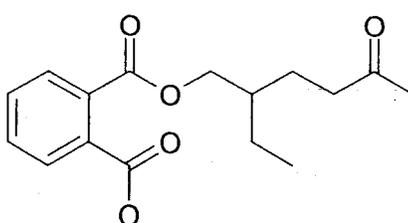
Metabolite V

MW: 308



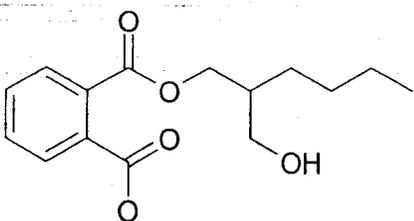
Metabolite VI

MW: 292



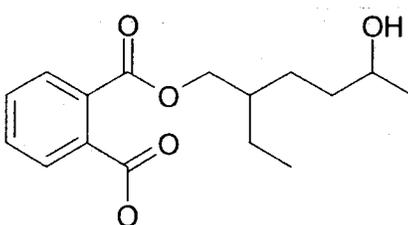
Metabolite VII

MW: 294



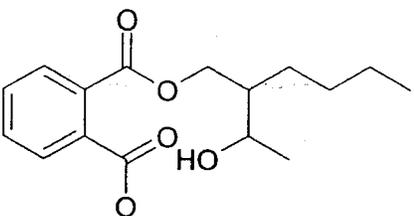
Metabolite IX

MW: 294



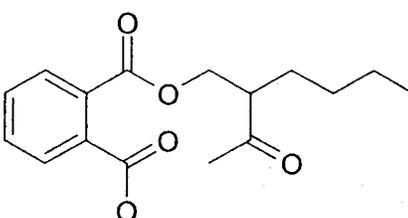
Metabolite XVII

MW: 294



Metabolite XXVI

MW: 292



MEHP :	mono-(2-ethylhexyl)phthalate
Metabolite I :	mono-(2-ethyl-3-carboxypropyl)phthalate
Metabolite II :	mono-(2-carboxyhexyl)phthalate
Metabolite IV :	mono-(2-[carboxymethyl]hexyl)phthalate
Metabolite V :	mono-(2-ethyl-5-carboxypentyl)phthalate
Metabolite VI :	mono-(2-ethyl-5-oxohexyl)phthalate
Metabolite VII :	mono-(2-[1-hydroxyethyl]hexyl)phthalate
Metabolite IX :	mono-(2-ethyl-5-hydroxyhexyl)phthalate
Metabolite XVII :	mono-(2-[2-hydroxyethyl]hexyl)phthalate
Metabolite XXVI :	mono-(2-[2-oxoethyl]hexyl)phthalate

4.4 Dose formulation

The test substance was prepared as a solution in corn oil one day before animal experimentation.

The radiochemical concentration of the stock [¹⁴C]-DEHP solution in acetone was determined by analysis in five replicate aliquots. [¹⁴C]-DEHP and unlabelled DEHP were combined at the appropriate ratio for the low and high dose to achieve the desired specific radioactivity.

The dose formulation was prepared separately for each dose by dispensing a known volume of stock liquid and adding a known volume of vehicle. The radiochemical concentration of these dose formulation was determined by analysis in five replicate aliquots.

4.5 Administration

The dose were formulated to deliver DEHP at either 200 mg/kg (102.5 µmoles/rat) or 1000 mg/kg (512.5 µmoles/rat) and approximately 1,000,000 dpm per animal in an individual dose volume of 5 ml/kg.

4.6 Treatment groups

Details of the treatment groups, types of samples and times of sampling are given below.

4.6.1 Blood radioactivity

4.6.1.1 Single oral dose

Two groups of 6 rats were selected to determine the blood radioactivity. Rats 1 to 6 received 200 mg/kg [¹⁴C]-DEHP, and rats 7 to 12 received 1000 mg/kg [¹⁴C]-DEHP with a specific radioactivity of 1,000,000 dpm. Immediately after administration, the rats were housed in groups of 3 in makrolon cages.

4.6.1.2 Repeated oral dose

Two groups of 15 rats were selected to investigate the effects on the blood radioactivity of a dose of [¹⁴C]-DEHP (specific radioactivity of 1,000,000 dpm) administered after a 5-day pre-treatment with unlabelled DEHP. Rats 7 to 21 received 200 mg/kg DEHP and rats 22 to 36 received 1000 mg/kg DEHP. Immediately after administration, the rats were housed in groups of 3 in makrolon cages.

4.6.2 Mass balance excretion study

4.6.2.1 Single oral dose

Two groups of 3 rats were allocated to the mass balance excretion study. Rats 1 to 3 received 200 mg/kg DEHP, and rats 4 to 6 received 1000 mg/kg DEHP with a specific radioactivity of 1,047,715 dpm.

Immediately after administration, the rats were housed individually in metabolism cages. Urine and faeces were collected at 24, 48, 72 and 96 hours after dosing.

4.6.2.2 Repeated oral dose

Two groups of 5 rats were selected to investigate the effect of multiple administration of [¹⁴C]-DEHP on the excretion of radioactive material. Rats received single daily doses of [¹⁴C]-DEHP (924,800 dpm/animal) for 10 consecutive days either at 200 mg/kg (number 2 to 6) or 1000 mg/kg (number 7 to 11).

Immediately after the first administration, the rats were housed individually in metabolism cages. Urine and faeces were collected at 24 hour intervals until the 13th day after the beginning of treatment.

4.7 Body weights

Body weights were recorded daily during pre-test and treatment period.

4.8 Sampling and storage

4.8.1 Identification

All samples were labelled with the rat number, time of sampling and identity of sample.

4.8.2 Urine and faeces

Urine and faeces were collected separately from the metabolism cage. The samples were kept cold during the collection periods by surrounding the collection vessels with dry ice. At the end of each study period the faecal pellets were removed from the metabolism cage. The cages were then carefully rinsed with distilled water and this was added to the urine.

Urine was diluted with distilled water to a 20ml final volume (or more if necessary), centrifuged, counted for ^{14}C and then stored at -40°C until further analysis.

Faeces were weighed, homogenised with distilled water (approximately a 20% homogenate) in a Potter homogeniser equipped with a teflon pestle, counted for ^{14}C and then stored at -40°C prior analysis.

4.8.3 Blood and plasma

Blood samples were taken from the orbital sinus of the animals and collected into tubes containing heparine.

After a single administration, rat numbers 1 to 3 and 7 to 9 were sampled 0.5, 1.5 and 24 hours after dosing and 4 to 6 and 10 to 12 were sampled 1, 4 and 48 hours after dosing.

After repeated administration, rat numbers 7 to 9 and 22 to 24 were sampled 0.5 and 48 hours after dosing, 10 to 12 and 25 to 27 were sampled 1 hours after dosing, 13 to 15 and 28 to 30 were sampled 1.5 hours after dosing, 16 to 18 and 31 to 33 were sampled 4 hours after dosing and 19 to 21 and 34 to 36 were sampled 24 hours after dosing.

Blood samples were collected, weighed, centrifuged (250 rpm, 10 min) and plasma samples were stored at -40°C until analysis.

4.9 Sample analysis

4.9.1 Radioactivity analysis

4.9.1.1 Dose solution

Five aliquots of each solution (100 μl) were counted in mini-vials using 4 ml of scintillation solution (Hisafe II).

4.9.1.2 Urine

Aliquots of urine (100 or 200 μl) were counted in triplicate in mini-vials using 4ml of scintillation solution.

4.9.1.3 Faeces

For quantification of total radioactivity, aliquots of homogenate faeces (400 μl) were placed in triplicate in glass scintillation vials. To these were added 1ml Soluene-350. The digestion was conducted for 2h at 50°C . The digest was allowed to cool to room temperature and 0.5ml isopropanol added. Bleaching was effected by the addition of 0.2ml hydrogen peroxide (30%). The vials were incubated at 50°C for 2h. After the addition of 5ml scintillation solution (Hionic Fluor, Packard), the radioactivity was determined one day later.

For quantification of [^{14}C]-labelled DEHP, one or 2.0 ml of homogenate faeces was added to 3.0ml of acetonitrile and 2.0ml of hexane. The mixture was sonicated and shaken for 20 min. After centrifugation at 1000g, the hexane phase was transferred to another tube, evaporated under nitrogen and counted for ^{14}C with 4 ml of scintillation solution (Hisafe II). This method for extraction of DEHP resulted in the recovery of 93% of ^{14}C -labelled DEHP.

4.9.1.4 Blood

Total plasma collected was counted in mini-vials using 2ml of scintillation solution (Hisafe II).

4.9.2 Gas chromatography analysis

4.9.2.1 Urine

Urine was acidified to pH2 with HCl 1M. After addition of 100µl of internal standard solution [5.0 mg/ml 4-[MHP] in methanol], urine was extracted three fold with diethyl-ether (urine/solvent, 2.5/5, v/v). The organic extract was dried with anhydrous Na₂SO₄, evaporated to dryness in a rotary evaporator and finally dissolved in methanol. This method resulted in the recovery of about 85% of total urinary ¹⁴C.

4.9.2.2 Faeces

One or 2.0 ml of homogenate faeces was added to 3.0ml of acetonitrile and 2.0ml of hexane. The mixture was sonicated and shaken for 20 min. After centrifugation at 1000g, the aqueous-acetonitrile phase was acidified to pH2 with HCl 1M and 100µl of internal standard solution [1.0 mg/ml DBP in methanol] was added. The mixture was extracted twice with 5ml of diethyl-ether. The combined organic phases were dried and the solvent was evaporated to dryness in a rotary evaporator. Finally, the residue was dissolved in methanol. This method for extraction gave a recovery of 95% of DEHP-derived metabolites.

4.9.2.3 Preparation of derivatives

Extraction residues of urine and faeces were subjected to derivatisation. Methyl esters were prepared by treatment with a freshly prepared diazomethane/ether solution (De Boer and Becker, 1954) for at least 2h at room temperature. Excess reagent was evaporated under nitrogen and the remaining residue dissolved in 50 or 100µl methanol before analysis by GC.

4.10 Pharmacokinetic data analysis

Pharmacokinetic data analysis were performed using the Innaphase KINETICA modelling program (Champs sur Marne, France). The non-compartmental analysis (NCA) was used to determine the toxicokinetic parameters. The area under the blood concentration-time curve (AUC) from time zero to the last data point was calculated by the trapezoidal method.

5. RESULTS

5.1 Pharmacokinetic study

5.1.1 Single dosing

5.1.1.1 Body weights

No effect on the body weight gain of female rats was observed following a single oral administration of 200 and 1000 mg/kg (table 1, appendix 1).

5.1.1.2 Recovery of radioactivity

5.1.1.2.1 Dose level 200 mg/kg

The mean concentration of radioactivity in blood (figure 1, table 2, appendix 2) increased from 42 ± 9 nmole DEHP-equivalents/g at 0.5 hours to a maximum of 64 ± 2 nmole DEHP-equivalents/g at 1.5 hours. The level then declined slowly to 34 ± 15 nmole DEHP-equivalents/g at 24 hours. No radioactivity was detected 48 hours after the administration.

The half-life of elimination was 7.1 hours and the area under the curve (AUC_{0-48h}) was 1426 nmole DEHP-equivalents.h.g⁻¹.

5.1.1.2.2 Dose level 1000 mg/kg

The mean concentration of radioactivity in blood (figure 1, table 2, appendix 2) increased from 226 ± 56 nmole DEHP-equivalents/g at 0.5 hours to a maximum of 353 ± 101 nmole DEHP-equivalents/g at 4 hours. The level then declined rapidly to 45 ± 11 nmole DEHP-equivalents/g at 24 hours and then slowly to 17 ± 11 nmole DEHP-equivalents/g at 48 hours.

The half-life of elimination was 10.2 hours and the AUC_{0-48h} was 5825 nmole DEHP-equivalents.h.g⁻¹.

5.1.2 Repeated dosing

5.1.2.1 Body weights

A slight and transient decreased of the body weight gain of female rats was observed following a 6-day repeated administration of 200 mg/kg/d. At 1000 mg/kg/d, no significant body weight gain was observed at the end of the observation period compared to beginning of treatment (table 3, appendixes 3 and 4).

5.1.2.2 Recovery of radioactivity

5.1.2.2.1 Dose level 200 mg/kg

Following a single oral administration of 200 mg/kg of [¹⁴C]-DEHP, after a 5-day pre-treatment with unlabelled DEHP, the mean concentration of radioactivity in blood (figure 2, table 4, appendix 5) increased from 24 ± 14 nmole DEHP-equivalents/g at 0.5 hours to a maximum of 77 ± 37 nmole

DEHP-equivalents/g at 1.5 hours. The level then declined rapidly to 7 ± 2 nmole DEHP-equivalents/g at 24 hours and then slowly to 2 ± 1 nmole DEHP-equivalents/g at 48 hours.

The half-life of elimination was 8.7 hours and the AUC_{0-48h} was 1007 nmole DEHP-equivalents.h.g⁻¹.

5.1.2.2.2 Dose level 1000 mg/kg

Following a single oral administration of 1000 mg/kg of [¹⁴C]-DEHP, after a 5-day pre-treatment with unlabelled DEHP, the mean concentration of radioactivity in blood (figure 2, table 4, appendix 5) increased from 271 ± 59 nmole DEHP-equivalents/g at 0.5 hours to a maximum of 405 ± 121 nmole DEHP-equivalents/g at 1.5 hours. The level then declined rapidly to 51 ± 2 nmole DEHP-equivalents/g at 24 hours and then slowly to 42 ± 32 nmole DEHP-equivalents/g at 48 hours.

The half-life of elimination was 13.5 hours and the AUC_{0-48h} was 6398 nmole DEHP-equivalents.h.g⁻¹.

5.2 Excretion and metabolism study

5.2.1 Single dosing

5.2.1.1 Body weights

A slight decrease of the body weight gain of female rats was observed following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP compared to the control group (table 5, appendix 6).

5.2.1.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces

5.2.1.2.1 Recovery of radioactivity

5.2.1.2.1.1 Dose level 200 mg/kg

The mean 0-96 hour recovery of radioactivity (as a percentage of radiochemical dose) was $57.6 \pm 17.0\%$ in urine and $31.3 \pm 6.7\%$ in faeces. The total mean recovery in excreta was $88.9 \pm 23.7\%$ (figure 3, table 6, appendixes 7 and 8).

During the first 24 hours, $48.9 \pm 14.5\%$ of the radiochemical dose were excreted in urine and $26.8 \pm 5.3\%$ in faeces (the total recovery was $75.7 \pm 19.8\%$). At the final collection period (72-96 hours) urine contained $1.0 \pm 0.4\%$ and faeces $0.3 \pm 0.2\%$ of the radiochemical dose.

5.2.1.2.1.2 Dose level 1000 mg/kg

The mean 0-96 hour recovery of radioactivity (as a percentage of radiochemical dose) was $47.4 \pm 12.6\%$ in the urine and $41.2 \pm 15.4\%$ in faeces. The total mean recovery in excreta was $88.6 \pm 6.9\%$ (figure 3, table 7, appendixes 7 and 8).

During the first 24 hours, $32.8 \pm 5.2\%$ of the radiochemical dose were excreted in urine and $26.2 \pm 15.0\%$ in faeces (the total recovery was $59.0 \pm 20.2\%$). At the final collection period (72-96 hours) urine contained $1.1 \pm 1.4\%$ and faeces $0.3 \pm 0.2\%$ of the radiochemical dose.

5.2.1.2.2 Analysis by gas chromatography

5.2.1.2.2.1 Dose level 200 mg/kg

Urine (figures 4 and 5, table 8, appendixes 9 and 10)

Expressed in % of the DEHP dose, MEHP-derived metabolites IX ($19.8 \pm 6.6\%$), VI ($11.4 \pm 3.2\%$), V ($10.5 \pm 3.3\%$) and VII ($3.8 \pm 1.6\%$) were the main compounds excreted in urine from female rats following a single oral administration of 200 mg/kg DEHP. Metabolites I, II, IV, XVII, and XXVI, were excreted at low levels (0.5-2.8% of the DEHP dose) in urine. DEHP and MEHP accounted for only $1.5 \pm 0.8\%$ and $1.8 \pm 1.2\%$, respectively.

DEHP, MEHP and MEHP-derived metabolites were mainly excreted during the first 24 hours. At the final collection period (48-72 hours), urine contained $2.2 \pm 2.0\%$ of the DEHP dose as DEHP, MEHP and MEHP-derived metabolites.

Metabolites from MEHP ω -1-oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII) represented 63 and 37% of the urinary MEHP-derived metabolites and 33.8 and 19.5% of the DEHP dose, respectively.

Faeces (figures 6 and 7, table 9, appendixes 11, 12 and 13)

Expressed in % of the DEHP dose, DEHP ($15.9 \pm 5.2\%$) and MEHP ($9.9 \pm 2.3\%$) were the main compounds identified in faeces from female rats following a single oral administration of 200 mg/kg DEHP. They represented 46 and 29% of the compounds excreted in faeces, respectively.

DEHP, MEHP and MEHP-derived were mainly excreted in the first 48 hours. At the final collection period (48-72 hours) faeces contained $2.3 \pm 0.04\%$ of the DEHP dose as DEHP, MEHP and MEHP-derived metabolites.

Metabolites from MEHP ω -1 oxidation (VI and IX) and ω -oxidation (V), represented 60 and 40% of the total of the MEHP-derived metabolites excreted in faeces, but only 5 and 3.3% of the DEHP dose.

Total excretion (figure 8)

After a single administration of 200 mg/kg DEHP to female rats, 61% of the DEHP dose were excreted as MEHP-derived metabolites in urine (53%) and faeces (8%). Sixteen percent of the dose were excreted as non-metabolised DEHP in faeces and 1% in urine. The excretion MEHP represented 10% and 2% of the administered DEHP dose in faeces and urine, respectively.

Metabolites from MEHP- ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII) represented 63 and 37% of the MEHP-derived metabolites and 39 and 23% of the DEHP dose, respectively.

5.2.1.2.2.2 Dose level 1000 mg/kg

Urine (figures 4 and 5, table 10, appendixes 9 and 10)

Expressed in % of the DEHP dose, MEHP-derived metabolites IX ($14.9 \pm 5.0\%$), VI ($11.3 \pm 4.9\%$), V ($8.9 \pm 3.2\%$) and VII ($4.0 \pm 1.6\%$) were the main compounds identified in urine from female rats following a single oral administration of 1000 mg/kg DEHP. Metabolites I, II, IV XVII and XXVI were excreted at low levels (0.3-2.0%) in the urine. DEHP and MEHP accounted for only $0.5 \pm 0.2\%$ of the dose.

DEHP, MEHP and MEHP-derived metabolites were mainly excreted during the first 24 hours. At the final collection period (48-72 hours) urine contained $3.5 \pm 4.3\%$ of the DEHP dose as DEHP, MEHP or

MEHP-derived metabolites.

Metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII) represented 62 and 38% of the total of the MEHP-derived metabolites and 28 and 17% of the DEHP dose, respectively.

Faeces (figures 6 and 7, table 11, appendixes 11, 12 and 13)

Expressed in % of the DEHP dose, DEHP ($29.6 \pm 14.1\%$) and MEHP ($6.4 \pm 3.5\%$) were the main compounds excreted in faeces following a single oral administration of 1000 mg/kg DEHP.

DEHP, MEHP and MEHP-derived metabolites were mainly excreted during the first 48 hours. At the final collection period (48-72 hours) faeces contained $1.9 \pm 1.5\%$ of the DEHP dose as DEHP, MEHP and MEHP-derived metabolites.

Metabolites from MEHP ω -1 oxidation (VI and IX) and ω -oxidation (V) represented 51 and 49% of the MEHP-derived metabolites and only 2.6 and 2.4% of the DEHP dose, respectively.

Total excretion (figure 8)

After a single administration of 1000 mg/kg DEHP to female rats, 50% of the DEHP dose were excreted as MEHP-derived metabolites in urine (45%) and faeces (5%). Thirty percent of the dose were excreted as non-metabolised DEHP in faeces and 0.5% in urine. The excretion MEHP represented 6% and 0.5% of the administered DEHP dose in faeces and urine, respectively.

Metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII) represented 61 and 39% of the MEHP-derived metabolites and 31 and 20% of the DEHP dose, respectively.

5.2.2 Repeated dosing

5.2.2.1 Body weights

The body weight gain was slightly delayed during the first 6 days of treatment with 200 mg/kg/d and throughout the whole treatment period with 1000 mg/kg/d (table 12, appendix 14).

5.2.2.2 Excretion of DEHP, MEHP and MEHP-derived metabolites in urine and faeces

5.2.2.2.1 Recovery of radioactivity

5.2.2.2.1.1 Dose level 200 mg/kg

Urine (figures 9 and 10, table 13, appendixes 15 and 16)

The mean recovery of radioactivity in urine was 67.5 ± 22.2 μ mole DEHP-equivalents/day during the treatment period (from 40.8 ± 23.0 μ mole DEHP-equivalents within 24 hours after the 1st administration up to 103.3 ± 18.5 μ mole DEHP-equivalents within 24 hours after the 6th administration). A slight decrease of the urinary excretion was observed after the 7th administration. After cessation of treatment, the daily excretion of radioactivity decreased rapidly, and only 8.0 ± 2.5 μ mole DEHP-equivalents were excreted during the last urine collection period (72-96 hours after the 10th administration).

The total recovery of radioactivity in urine was $68.7 \pm 21.4\%$ of the total administered dose.

Faeces (figures 9 and 10, table 14, appendixes 15 and 16)

The mean recovery of radioactivity in faeces was 22.4 ± 8.1 $\mu\text{mole DEHP-equivalents/day}$ during the treatment period (from 10.8 ± 9.9 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 1st administration up to 35.8 ± 6.6 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 8th administration). The faecal excretion increased until the 8th administration and then decreased thereafter. After cessation of treatment, the daily excretion of radioactivity decreased rapidly, and only 1.1 ± 0.4 $\mu\text{mole DEHP-equivalents}$ were excreted during the last collection period (72-96 hours after the 10th administration).

The total recovery of radioactivity in faeces was $23.2 \pm 5.4\%$ of the total administered dose.

Total excretion

The mean recovery of radioactivity in excreta was 90.0 ± 22.1 $\mu\text{mole DEHP-equivalents/day}$ during the treatment period (from 51.6 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 1st administration up to 127.2 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 6th administration). A slight decrease of the excretion was observed after the 8th administration. After cessation of treatment, the daily excretion of radioactivity decreased rapidly, and only 9.1 $\mu\text{mole DEHP-equivalents}$ were excreted during the last excreta collection period (72-96 hours after the 10th administration).

The total recovery of radioactivity in excreta was $91.9 \pm 18.2\%$ of the total administered dose.

5.2.2.2.1.2 Dose level 1000 mg/kg

Urine (figures 11 and 12, table 15, appendixes 17 and 18)

The mean recovery of radioactivity in urine was 341.2 ± 51.5 $\mu\text{mole DEHP-equivalents/day}$ during the treatment period (from 204.2 ± 28.4 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 1st administration up to 380.2 ± 120.9 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 9th administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly, and only 45.3 ± 31.3 $\mu\text{mole DEHP-equivalents}$ were excreted during the last urine collection period (72-96 hours after the 10th administration).

The total recovery of radioactivity in urine was $73.4 \pm 7.1\%$ of the total administered dose.

Faeces (figures 11 and 12, table 12, appendixes 17 and 18)

The mean recovery of radioactivity in faeces was 135.3 ± 29.5 $\mu\text{mole DEHP-equivalents/day}$ during the treatment period (from 129.0 ± 73.0 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 1st administration up to 189.9 ± 108.1 $\mu\text{mole DEHP-equivalents}$ within 24 hours after the 5th administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly, and only 3.5 ± 1.3 $\mu\text{mole DEHP-equivalents}$ were excreted during the last faeces collection period (72-96 hours after the 10th administration).

The total recovery of radioactivity in faeces was $27.8 \pm 7.0\%$ of the total administered dose.

Total excretion

The mean recovery of radioactivity in excreta was 476.5 ± 57.3 μ mole DEHP-equivalents/day during the treatment period (from 333.2 μ mole DEHP-equivalents within 24 hours after the 1st administration up to 531.3 μ mole DEHP-equivalents within 24 hours after the 5th administration). After cessation of treatment, the daily excretion of radioactivity decreased rapidly, and only 48.8 μ mole DEHP-equivalents were excreted during the last excreta collection period (72-96 hours after the 10th administration).

The total recovery of radioactivity in excreta was $101.2 \pm 10.7\%$ of the total administered dose.

5.2.2.2.2 Analysis by gas chromatography

5.2.2.2.2.1 Dose level 200 mg/kg

Urine (Figure 13, table 17, appendixes 19 and 20)

Within 24 hours after the 1st, 4th, 7th and 10th administration of 200 mg/kg/d, DEHP was excreted in urine from female pregnant rats at levels of 0.46 ± 0.56 , 2.04 ± 1.06 , 1.87 ± 0.94 and 0.33 ± 0.36 μ moles. These quantities represented only 1.1, 2.4, 3.1 and 0.7% of the compounds excreted in urine, respectively.

MEHP levels of 0.47 ± 0.18 , 1.34 ± 0.16 , 1.18 ± 0.83 and 0.65 ± 0.50 μ moles were recovered in urine from female rats within 24 hours after the 1st, 4th, 7th and 10th administration of 200 mg/kg/d, respectively. These quantities represented only 1.1, 1.6, 2.0 and 1.4% of the compounds excreted.

MEHP-derived metabolites were excreted at levels of 39.8, 82.1, 56.6 and 46.7 μ moles after the 1st, 4th, 7th and 10th administration, respectively. These levels represented 98, 96, 95 and 98% of the total excretion, respectively.

Metabolites from MEHP ω -1 oxidation (VI, IX, XVII, XXVI) were excreted at levels of 24.6, 50.0, 38.9 and 30.3 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 60, 58, 65 and 64% of the total excretion or 62, 61, 69 and 65% of the MEHP-derived metabolites excreted in urine after the 1st, 4th, 7th and 10th administration, respectively.

Metabolites from MEHP ω -oxidation (I, II, IV, V and VII) were excreted at levels of 15.2, 32.1, 17.7 and 16.3 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 37, 38, 30, 34% of the total excretion or 38, 39, 31 and 35% of the MEHP-derived metabolites excreted in urine after the 1st, 4th, 7th and 10th administration, respectively.

Faeces (figure 14, table 18, appendixes 21, 23 and 24)

DEHP, MEHP and MEHP-derived metabolites VI+IX were the main compounds recovered in faeces from female rats following a repeated oral administration of 200 mg/kg/d DEHP.

The part of DEHP in the faecal excretion decreased dramatically with time, from 7.27 ± 0.3 μ moles (40% of the total excretion) after the 1st to 0.45 ± 0.34 μ moles (2.5% of the total excretion) after the 10th administration.

The faecal excretion of MEHP was stable with mean values of 6.42 ± 0.82 , 6.10 ± 2.61 , 8.14 ± 2.98 and 5.84 ± 2.13 μ moles after the 1st, 4th, 7th and 10th administration (32-36% of the total excretion), respectively.

The recovery of the MEHP-derived metabolites increased with the number of administrations and levels of 4.2, 9.4, 14.3 and 11.6 μ moles were excreted within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 23, 49, 56 and 65% of the compounds excreted after the 1st, 4th, 7th and 10th administrations, respectively.

Metabolites from MEHP ω -1 oxidation (VI and IX) were excreted at levels of 2.8, 5.3, 8.4 and 6.6 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 68, 57, 59 and 57% of the MEHP-derived metabolites or 16, 28, 33 and 37% of the total excretion in urine after the 1st, 4th, 7th and 10th administration, respectively.

Metabolites from MEHP ω -oxidation (I, IV, V and VII) were excreted at levels of 1.4, 4.0, 5.9 and 5.0 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 32, 43, 41 and 43% of the MEHP-derived metabolites or 8, 21, 23 and 28% of the total excretion in urine after the 1st, 4th, 7th and 10th administration, respectively.

Total excretion (figure 15)

The excretion of non-metabolised DEHP decreased significantly from 7.73 μ moles after the 1st administration to 5.71 and 5.11 μ moles after the 4th and 7th administration and to 0.78 μ moles after the 10th administration. DEHP represented 14, 5, 6 and 1% of the total excretion within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. DEHP was essentially excreted in faeces after the 1st administration (94%), then the part of the faecal excretion of DEHP represented only 64% after the 4th and 7th administration and 58% after the 10th administration, respectively.

Within 24 hours after the 1st, 4th, 7th and 10th administration, the excretion of MEHP was 6.89, 7.44, 9.32 and 6.49 μ moles, respectively. These quantities represented respectively 12, 7, 11 and 10% of the total excretion and 82 to 93% of MEHP were excreted in faeces.

MEHP-derived metabolites were excreted at levels of 44.0, 91.4, 71.0 and 58.3 μ moles per day after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 75, 87, 83 and 89% of the total excretion after the 1st, 4th, 7th and 10th administration, respectively. Ninety percent after the 1st and the 4th administrations and 80% after the 7th and 10th administrations of the MEHP-derived metabolites were excreted in urine.

The total excretion of metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) was 27.4, 55.3, 47.3 and 37.0 μ moles per day and represented 62, 60, 67 and 63% of the MEHP-derived metabolites excreted after the 1st, 4th, 7th and 10th administrations, respectively. Ninety percent of these metabolites were excreted in urine after the 1st and 4th administration and 82% after the 7th and 10th administration.

The total excretion of metabolites from MEHP ω -oxidation (I, II, IV, V and VII) was 16.6, 36.2, 23.1 and 21.3 μ moles per day and represented 38, 40, 33, 37% of the MEHP-derived metabolites excreted after the 1st, 4th, 7th and 10th administrations, respectively. Ninety-two and 89% of these metabolites were excreted in urine after the 1st and 4th administration, respectively and 77% after the 7th and 10th administration.

5.2.2.2.2 Dose level 1000 mg/kg

Urine (Figure 16, table 19, appendixes 19 and 20)

Within 24 hours after the 1st, 4th, 7th and 10th administration of 1000 mg/kg/d, DEHP was excreted in urine from female pregnant rats at levels of 0.28 ± 0.31 , 5.86 ± 3.39 , 2.30 ± 1.46 and 2.36 ± 1.42 μ moles. These quantities represented only 0.1, 1.4, 0.6 and 0.6% of the compounds excreted in urine from female rats within 24 hours after the 1st, 4th, 7th and 10th administration, respectively.

Within 24 hours after the 1st, 4th, 7th and 10th administration, MEHP was excreted at levels of 1.54 ± 1.71 , 16.87 ± 3.91 , 8.20 ± 2.59 and 4.34 ± 2.49 μ moles, respectively. These quantities represented only 0.7, 4.1, 2.1 and 1.2% of the compounds excreted in urine, respectively.

MEHP-derived metabolites were excreted at levels of 202.4, 387.2, 379.8 and 362.9 μ moles after the 1st, 4th, 7th and 10th administration, respectively. These levels represented 99, 94, 97 and 98% of the total excretion, respectively.

Metabolites from MEHP ω -1 oxidation (VI, IX, XVII, XXVI) were excreted at levels of 143.0, 263.2, 282.7 and 267.7 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 70, 64, 72 and 72% of the total excretion or 71, 68, 74 and 74% of the totality of the MEHP-derived metabolites excreted in urine within 24 hours after the 1st, 4th, 7th and 10th administrations, respectively.

Metabolites from MEHP ω -oxidation (I, II, IV, V and VII) were excreted at levels of 59.4, 124.0, 97.2 and 95.2 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 29, 30, 25 and 26% of the total excretion or 29, 32, 26 and 26% of the totality of the MEHP-derived metabolites, respectively.

Faeces (figure 17, table 20, appendixes 22, 23 and 24)

DEHP, MEHP and MEHP-derived metabolites VI+IX were the main compounds excreted in faeces from female rats following repeated oral administration of 1000 mg/kg/d DEHP.

The part of DEHP in the total faecal excretion decreased significantly with the repetition of the administration, from 85% (110.61 \pm 64.75 μ moles) and 63% (101.84 \pm 117.97 μ moles) after the 1st and 4th administration to 25% (30.54 \pm 13.5 μ moles) and 31% (34.85 \pm 50.86 μ moles) after the 7th and 10th administration, respectively.

MEHP was excreted at levels of 15.74 \pm 8.87, 38.39 \pm 14.46, 38.76 \pm 7.98 and 37.01 \pm 32.17 μ moles per day after the 1st, 4th, 7th and 10th administration. These quantities represented 12, 24, 31 and 33% of the total excretion, respectively.

The recovery of the MEHP-derived metabolites increased with the number of administrations and levels of 2.5, 22.5, 53.8 and 40.22 μ moles were excreted within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 2, 14, 44 and 36% of the total excretion in urine after the 1st, 4th, 7th and 10th administrations, respectively.

Metabolites from MEHP ω -1 oxidation (VI and IX) were excreted at levels of 1.3, 8.5, 31.2 and 21.0 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 53, 38, 58 and 52% of the MEHP-derived metabolites or 1, 5, 25 and 19% of the total excretion in urine after the 1st, 4th, 7th and 10th administration, respectively.

Metabolites from MEHP ω -oxidation (I, IV, V and VII) were excreted at levels of 1.2, 14.0, 22.6 and 19.2 μ moles within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. These metabolites represented 47, 62, 42 and 48% of the MEHP-derived metabolites or 1, 9, 18 and 17% of the total excretion in urine after the 1st, 4th, 7th and 10th administration, respectively.

Total excretion (figure 18)

The excretion of non-metabolised DEHP decreased significantly from 110.9 and 107.7 μ moles after the 1st and 4th administration to 32.8 and 37.2 μ moles after the 7th and 10th administration, respectively. DEHP represented 33, 19, 6 and 8% of the total excretion within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. DEHP was almost totally excreted in faeces after the 1st administration (>99%), then the part of the faecal excretion of DEHP represented about 94% after the 4th administration.

Within 24 hours after the 1st, 4th, 7th and 10th administration, the excretion of MEHP was 17.3, 55.3, 47.0 and 41.3 μ moles, respectively. These quantities represented 5, 10, 9 and 9% of the total excretion and 91, 69, 82 and 89% of MEHP were excreted in faeces, respectively.

MEHP-derived metabolites were excreted at levels of 204.9, 409.7, 433.7 and 403.1 μ moles per day after the 1st, 4th, 7th and 10th administration, respectively. The part of these metabolites in the total excretion increased with time and represented 61, 71, 84 and 84% after the 1st, 4th, 7th and 10th administrations, respectively. Ninety-nine, 95, 88 and 90% percent of these metabolites were excreted

in urine after the 1st, 4th, 7th and 10th administration.

The total excretion of metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) was 144.4, 271.6, 313.9 and 288.7 μ moles per day and represented 70, 66, 72 and 72% of the MEHP-derived metabolites excreted after the 1st, 4th, 7th and 10th administrations, respectively. Ninety-nine, 97, 90 and 93 percent of these metabolites were excreted in urine after the 1st, 4th, 7th and 10th administration, respectively.

The total excretion of metabolites from MEHP ω -oxidation (I, II, IV, V and VII) was 60.6, 138.0, 119.8 and 114.4 μ moles per day and represented 30, 34, 28 and 28% of the MEHP-derived metabolites excreted after the 1st, 4th, 7th and 10th administrations, respectively. Ninety-eight, 90, 81 and 83% of these metabolites were excreted in urine after the 1st, 4th, 7th and 10th administration.

Metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII), represented 70, 66, 72 and 72% and 30, 34, 28 and 28% of the total of the MEHP-derived metabolites excreted within 24 hours after the 1st, 4th, 7th and 10th administrations, respectively.

6. DISCUSSION

The absorption of radioactive material following single doses of [^{14}C]-DEHP at 200 mg/kg and 1000 mg/kg to female Wistar rats was rapid. The maximum blood concentrations (C_{max}), approximately 64 and 353 nmole DEHP-equivalents/g for the low and the high doses, were obtained 1.5 and 4 hours after dosing, respectively. The half-life of elimination were approximately 7.1 and 10.2 hours at the low and the high dose level, respectively. The areas under the curves ($\text{AUC}_{0-48\text{h}}$) were 1426 and 5825 nmole DEHP-equivalents.h.g $^{-1}$ for 200 mg/kg and 1000 mg/kg dose levels respectively. This 4.1-fold difference in AUC is consistent with the blood profile in which there is a 5.5-fold difference in the maximum concentration between dose levels and the 5-fold difference in dose levels. After 48 hours, no radioactivity was detected in blood samples following 200 mg/kg dose, and a level representing 5% of the C_{max} was still present following 1000 mg/kg dose.

After a 5-day pre-treatment of female rats with unlabelled DEHP at 200 or 1000 mg/kg bw/d, the absorption of radioactive material following a single dose of [^{14}C]-DEHP at 200 mg/kg and 1000 mg/kg was rapid. The maximum blood concentrations (C_{max}), approximately 77 and 405 nmole DEHP-equivalents/g for the low and the high doses, were obtained 1.5 hours after dosing, respectively. The half-life of elimination were approximately 8.7 and 13.5 hours at the low and the high dose level, respectively. The $\text{AUC}_{0-48\text{h}}$ were 1007 and 6398 nmole DEHP-equivalents.h.g $^{-1}$ for 200 mg/kg and 1000 mg/kg dose levels respectively. This 6.3-fold difference in AUC is still consistent with the blood profile in which there is a 5.2-fold difference in the maximum concentration between dose levels and the 5-fold difference in dose levels. After 48 hours, a very low level of radioactivity was detected in blood samples following the 200 mg/kg dose, and a level representing 10% of the C_{max} was still present following the 1000 mg/kg dose.

According to the previous results, and whatever the dose level, a 5-day pre-treatment with unlabelled DEHP did not affect significantly the $\text{AUC}_{0-48\text{h}}$ of a further [^{14}C]-DEHP dose. However, the half-life of elimination at the low and high dose level increased from 7.1 and 10.2 hours after a single administration to 8.7 and 13.5 hours after a repeated administration, respectively.

After a single oral administration, excretion of radioactive material was very rapid as most was excreted in the first 48 hours. Following the low and high dose administrations, within 96 hours approximately 58% and 47% of the dose were recovered in the urine and approximately 31% and 41% in the faeces, respectively. The total overall mean recoveries were approximately 89% at both dose levels. Approximately 17% and 30% of the low and high DEHP dose were excreted unchanged almost totally in faeces, either due to a non absorption or/and a re-excretion as a consequence of an entero-hepatic re-circulation. MEHP, the proximate metabolite issued from DEHP hydrolysis, was also almost totally excreted in faeces (12% and 7% of the DEHP dose at 200 mg/kg and 1000 mg/kg, respectively). MEHP-derived metabolites were mainly excreted in urine (53% and 45% of the DEHP dose at 200 and 1000 mg/kg, respectively) and a small part in faeces (8% and 5% of the dose at 200 mg/kg and 1000 mg/kg, respectively). Total excretion of MEHP-derived metabolites reached 61 and 50% of the dose at 200 mg/kg and 1000 mg/kg, respectively. According to the blood concentrations observed, such difference could be related to a saturation of the oxidation pathways rather than a saturation of DEHP absorption or/and DEHP hydrolysis. Whatever the dose level, MEHP ω -1 oxidation was the main metabolic pathway (about 60%), followed by ω -oxidation (about 40%).

During a 10-day repeated administration of [^{14}C]-DEHP, the urine and faecal excretion reached a steady-state after 4 days. Approximately 69% and 73% of the total [^{14}C]-DEHP dose were recovered in the urine and 23% and 28% in the faeces at 200 and 1000 mg/kg/d, respectively. At 200 mg/kg/d, the excretion in urine decreased slightly after the 7th administration and increased in faeces from the 1st administration. No such effect was observed at 1000 mg/kg/d. The total overall mean recovery of radioactive material in excreta was similar to that obtained following a single administration at 200 mg/kg/d, approximately 92% and slightly higher at 1000 mg/kg/d, approximately 101%. These values of total excretion and the observed steady-state excluded any significant bio-accumulation of DEHP.

During the repeated administration of 200 mg/kg bw/d DEHP for 10 days to female rats, the excretion of non-metabolised DEHP decreased significantly and represented 14, 5, 6 and 1% of the total excretion within 24 hours after the 1st, 4th, 7th and 10th administration, respectively. DEHP was essentially excreted in faeces after the 1st administration (94%), then the part of the faecal excretion of DEHP represented only 64% after the 4th and 7th administration and 58% after the 10th administration, respectively. The excretion of MEHP was stable and represented respectively 12, 7, 11 and 10% of the total excretion within 24 hours after the 1st, 4th, 7th and 10th administration. Eighty-two to 93% of MEHP were excreted in faeces. The excretion of MEHP-derived metabolites increased slightly with time and represented 75, 87, 83 and 89% of the total excretion after the 1st, 4th, 7th and 10th administration, respectively. Ninety percent after the 1st and the 4th administrations and 80% after the 7th and 10th administrations of these metabolites were recovered in urine. The total excretion of metabolites issued from ω -1 oxidation (VI, IX, XVII and XXVI) or ω -oxidation (I, II, IV, V and VII) represented 62, 60, 67 and 63% or 38, 40, 33, 37% of the MEHP-derived metabolites excreted after the 1st, 4th, 7th and 10th administrations, respectively.

During the repeated administration of 1000 mg/kg bw/d DEHP for 10 days to female rats, the excretion of non-metabolised DEHP decreased significantly from 33 and 19% of the total excretion after the 1st and 4th administration to 6 and 8% after the 7th and 10th administration, respectively. DEHP was almost totally excreted in faeces after the 1st administration (>99%), then the part of the faecal excretion of DEHP represented about 94% after the 4th administration. Within 24 hours after the 1st, 4th, 7th and 10th administration, the excretion of MEHP was stable and represented 5, 10, 9 and 9% of the total excretion and 91, 69, 82 and 89% of MEHP were excreted in faeces, respectively. The part of MEHP-derived metabolites in the total excretion increased slightly with time and represented 61, 71, 84 and 84% after the 1st, 4th, 7th and 10th administrations, respectively. Ninety-nine, 95, 88 and 90% percent of these metabolites were excreted in urine after the 1st, 4th, 7th and 10th administration. Metabolites from MEHP ω -1 oxidation (VI, IX, XVII and XXVI) and ω -oxidation (I, II, IV, V and VII), represented 70, 66, 72 and 72% and 30, 34, 28 and 28% of the total of the MEHP-derived metabolites excreted within 24 hours after the 1st, 4th, 7th and 10th administrations, respectively.

In conclusion, [¹⁴C]-DEHP was rapidly, extensively and dose-related absorbed following a single or repeated oral administration to female Wistar rats as mirrored by the blood concentration curve profile and a rapid excretion in urine and faeces. A 5-day pre-treatment did not have any significant effect on the total absorption rate but increased slightly the half-life of elimination at the high dose level. After a single or a repeated administration, [¹⁴C]-DEHP was excreted very quickly in excreta and the recovery reached or exceeded 90% of the administered dose. [¹⁴C]-DEHP was excreted in majority as MEHP-derived metabolite essentially in urine, as MEHP mainly in faeces and DEHP almost totally in faeces. Omega-1 oxidation was the main metabolic pathway (c.a. 60-70%) of the production of MEHP-derived metabolite. The repeated administration was characterised by a decrease of the DEHP excretion and a concomitant increase of the MEHP-derived metabolites but without alteration of the ω/ω -1 oxidation ratio. This effect was probably related to the metabolic activation which took place after a few days of treatment.

7. REFERENCES

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FIGURES

Pharmacokinetic study

Figure 1. Concentration of radioactivity in blood (in nmole equivalent of DEHP per gram of blood) from female rats following a single oral administration of [¹⁴C]-DEHP at 200 or 1000 mg/kg.

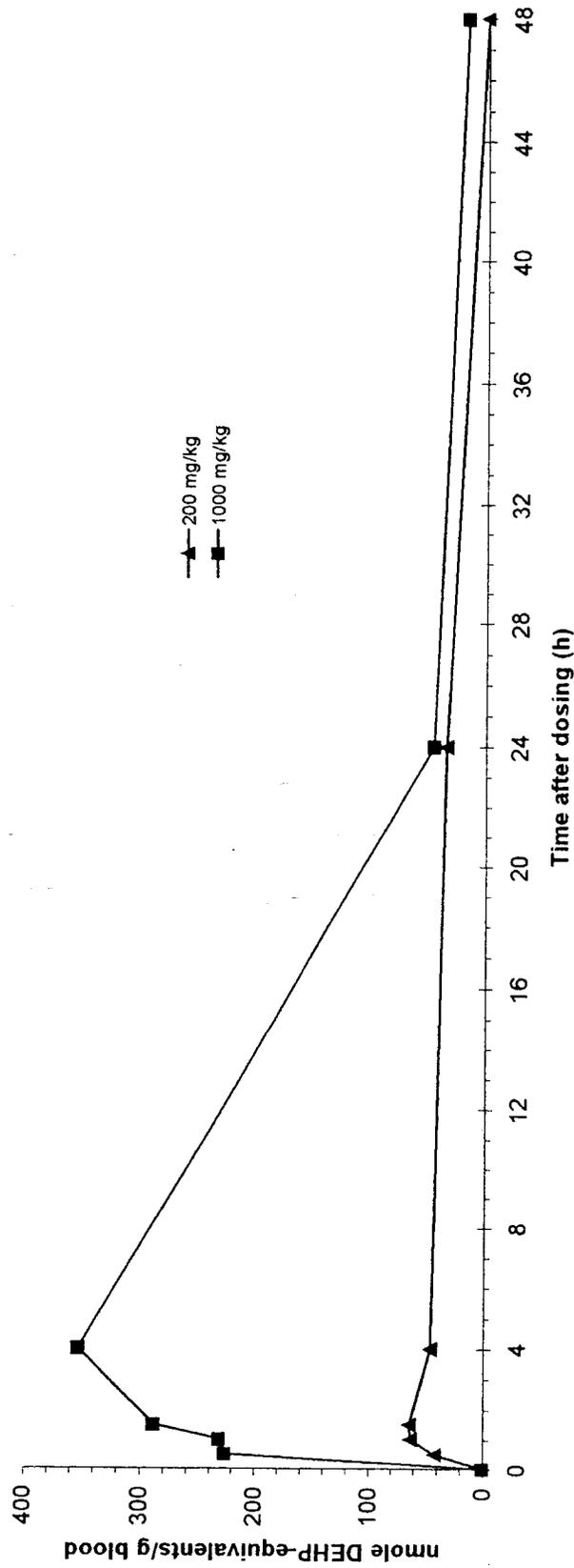
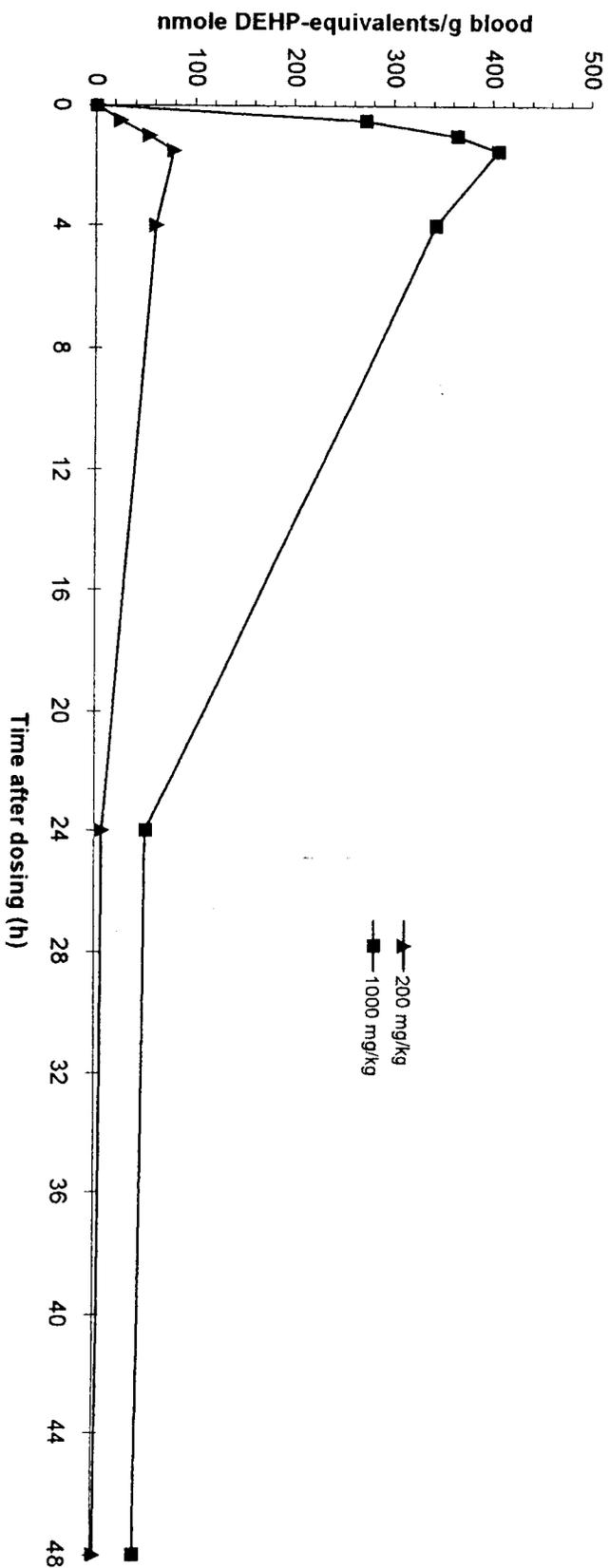


Figure 2. Concentration of radioactivity in blood (in nmole equivalent of DEHP per gram of blood) from female rats following a single oral administration of 200 or 1000 mg/kg [¹⁴C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled-DEHP, respectively.



Excretion and metabolism study, single dosing.

Figure 3. Recovery of radioactivity (in % of the DEHP dose) in urine and faeces from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg [¹⁴C]-DEHP.

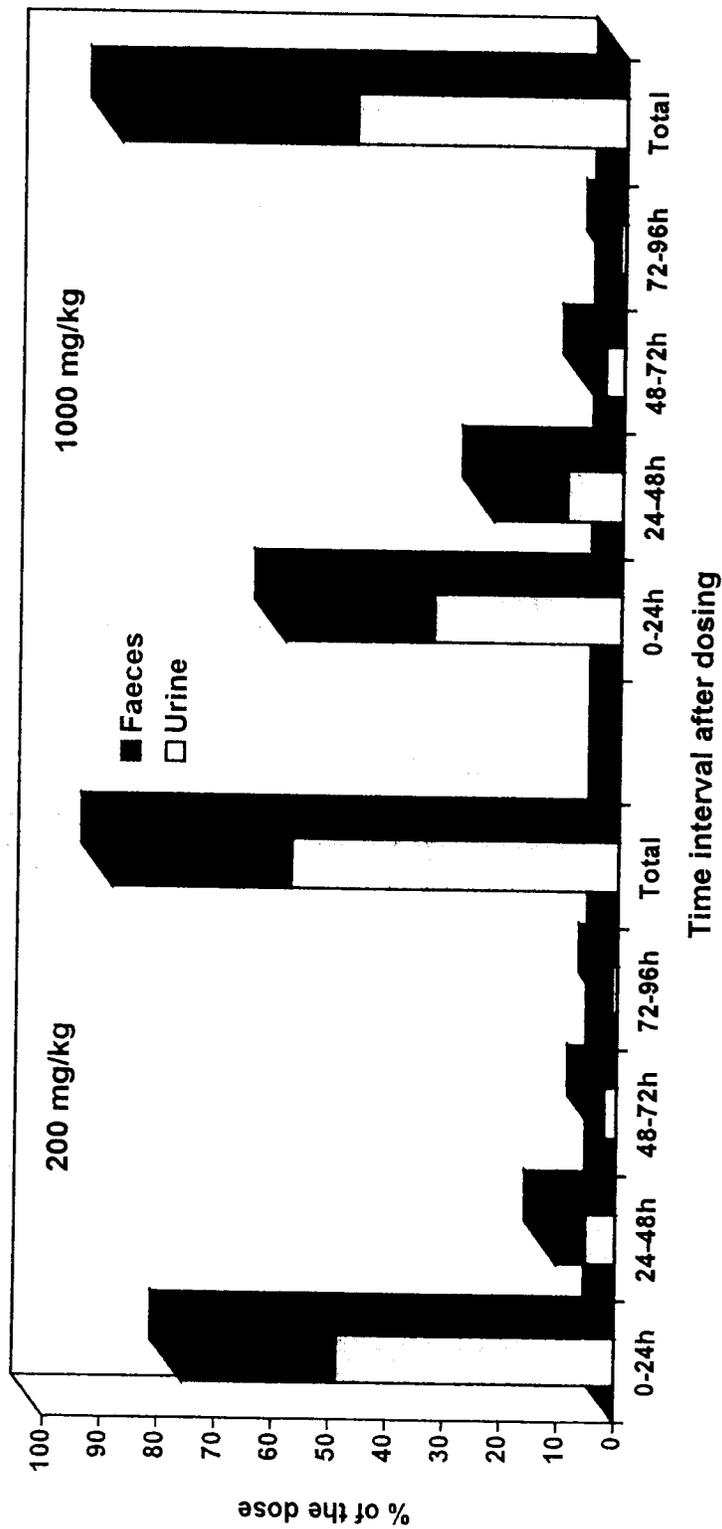


Figure 4. Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

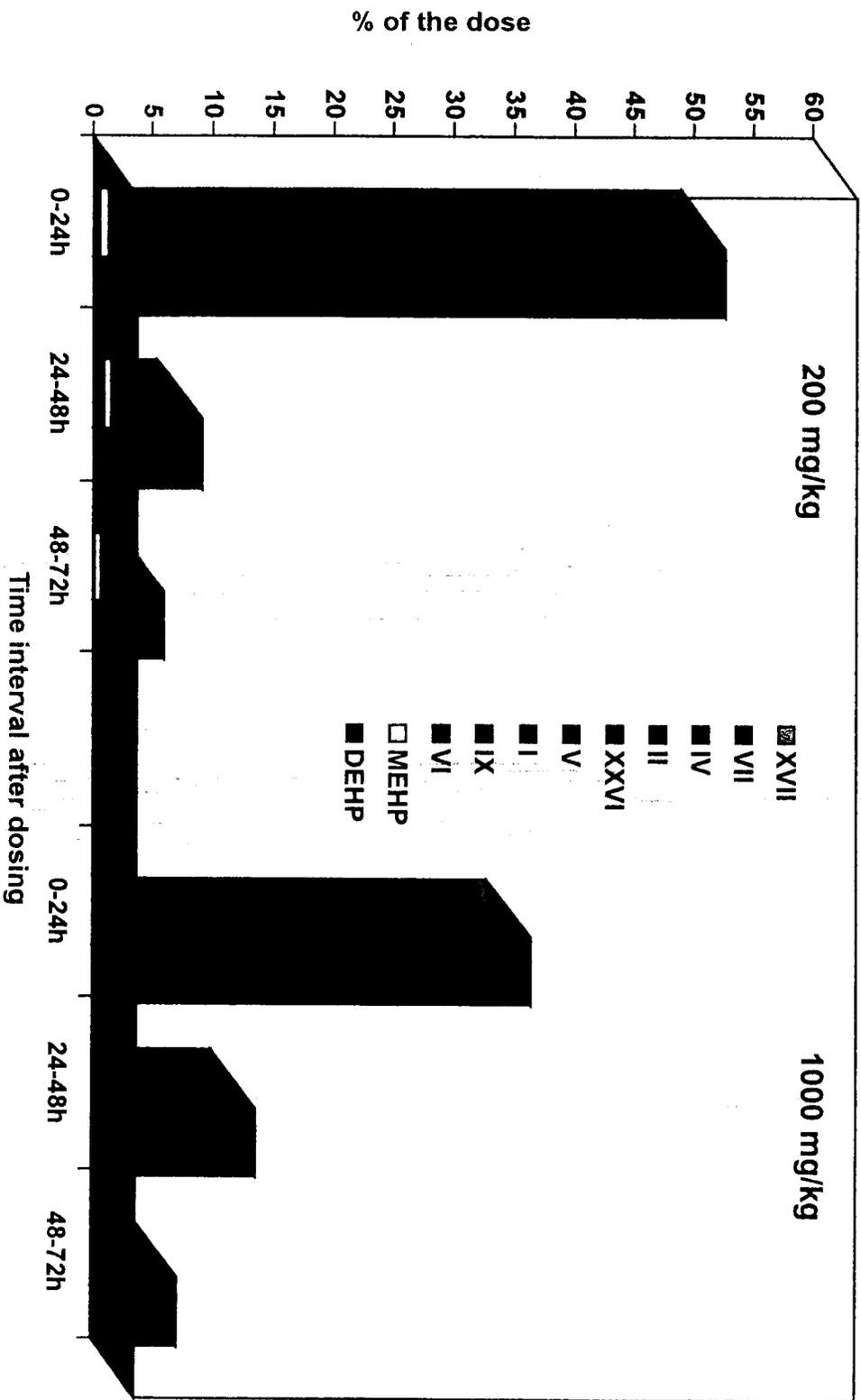


Figure 5. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in urine from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

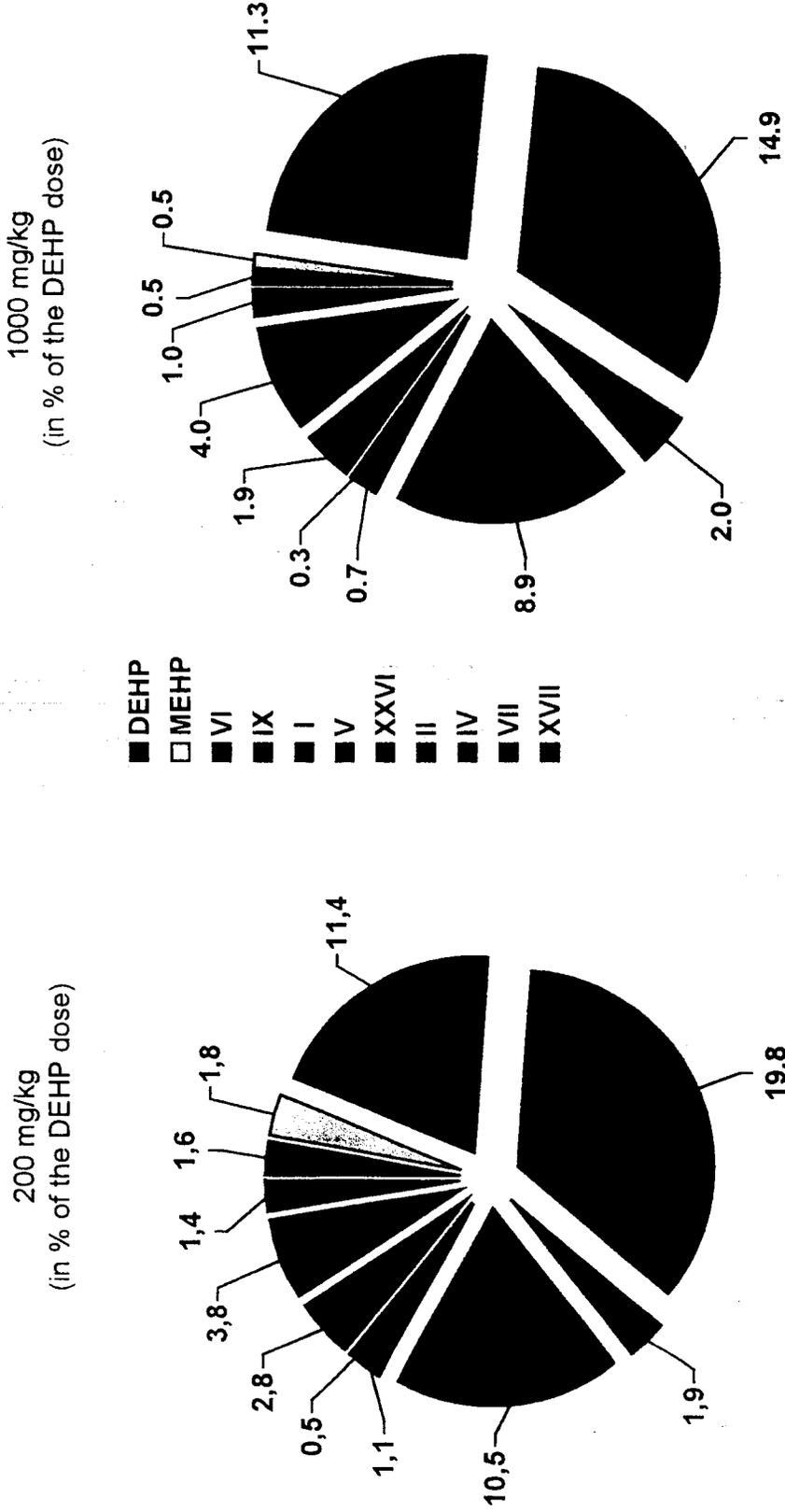


Figure 6. Recovery of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

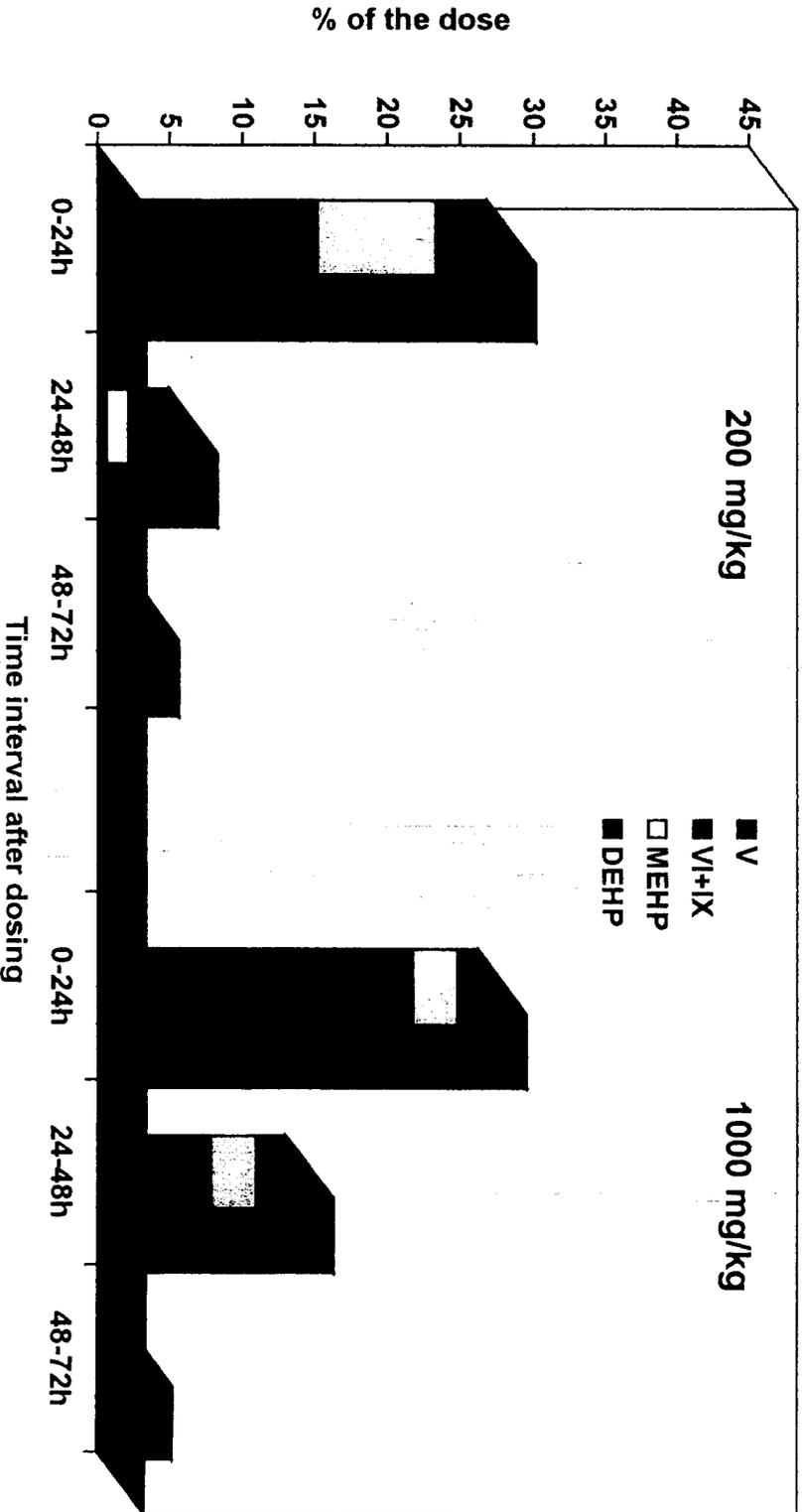


Figure 7. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in faeces from female rats following a single oral administration of 200 mg/kg or 1000 mg/kg DEHP.

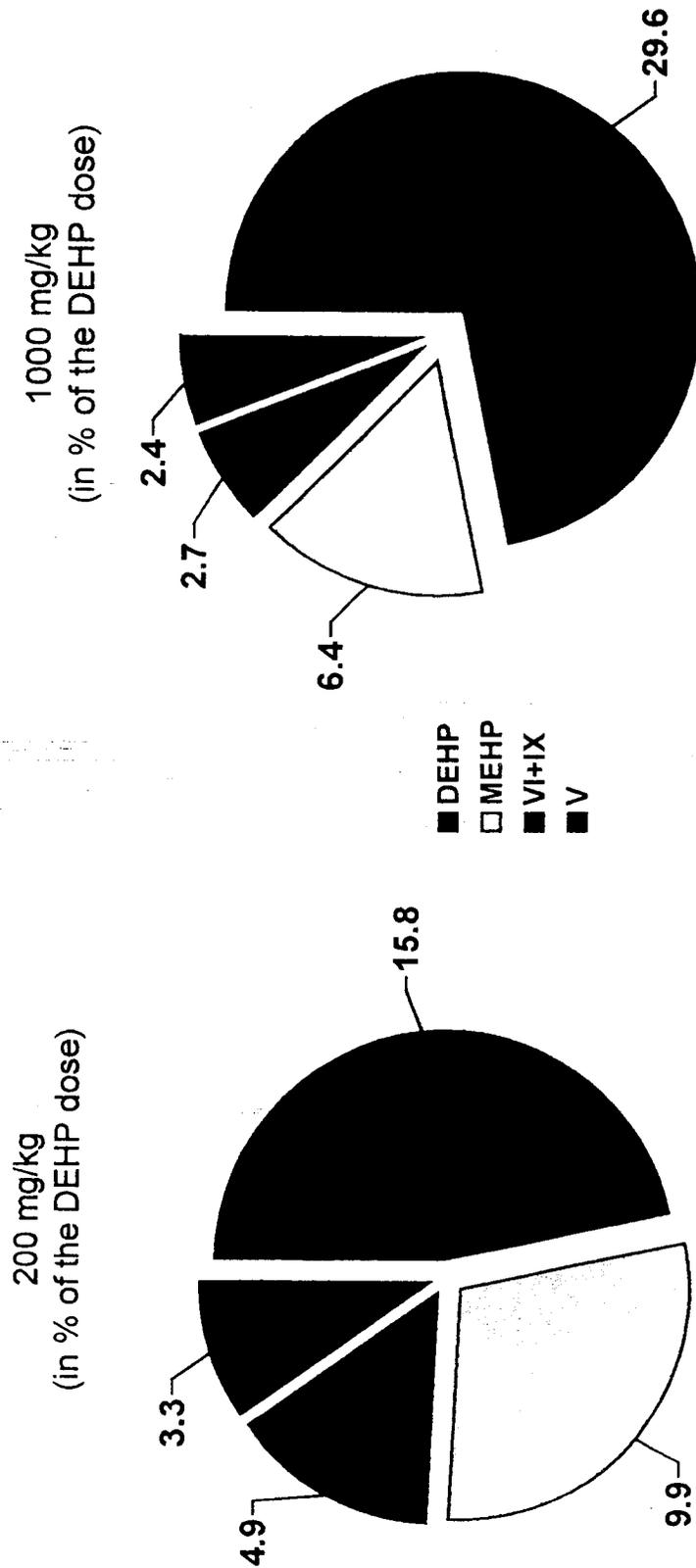
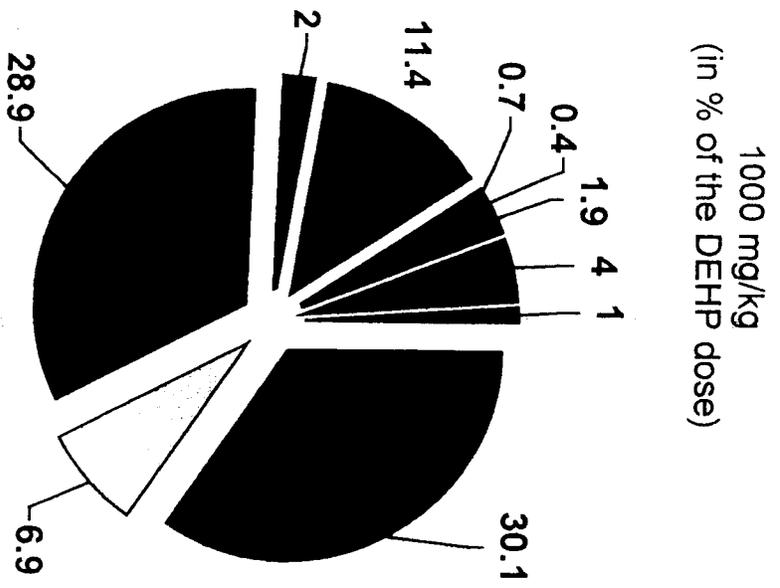
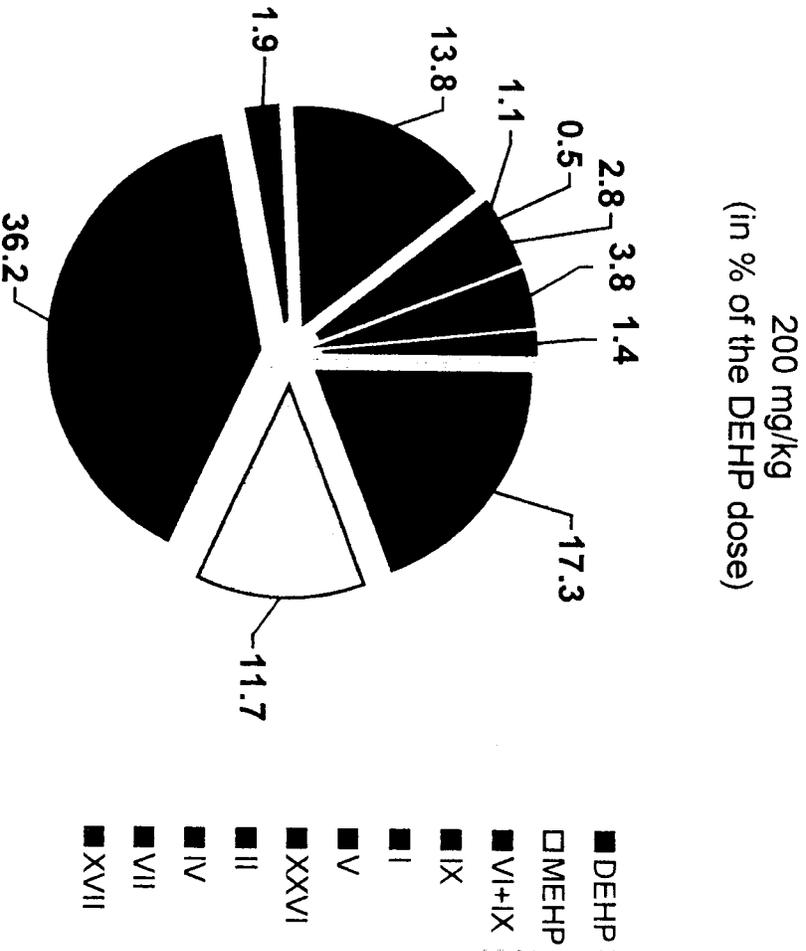


Figure 8. Cumulative excretion (0-72h) of DEHP, MEHP and MEHP-derived metabolites (in % of the DEHP dose) in the urine and faeces of female rats treated with a single oral dose of 200 mg/kg or 1000 mg/kg DEHP.



- DEHP
- MEHP
- VI+IX
- IX
- I
- V
- XXVI
- II
- IV
- VII
- XVIII

Excretion and metabolism study, repeated dosing.

Figure 9. Mean recovery of radioactivity (in $\mu\text{mole DEHP-equivalents}$) in urine and faeces from female rats collected within 24 hours following the 1st to the 10th oral administration of 200 mg/kg/d [^{14}C]-DEHP.

Rats received a 10-day repeated oral administration of 102.5 $\mu\text{moles/animal/d}$ of [^{14}C]-DEHP with a specific activity of 924800 dpm.

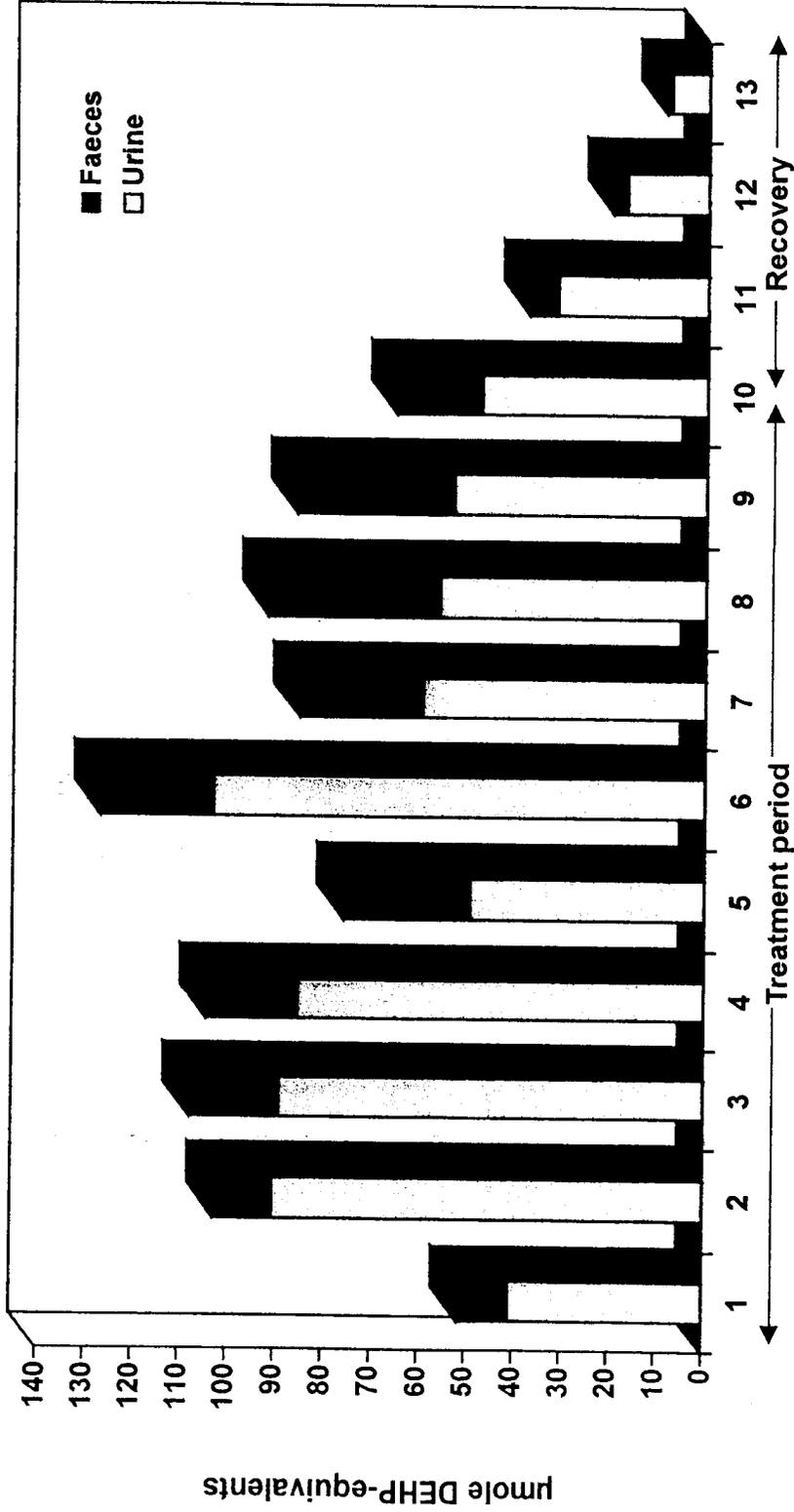


Figure 10. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female rats collected within 24 hours following the 1st to the 10th oral administration of 200 mg/kg/d [¹⁴C]-DEHP.

Rats received a 10-day repeated oral administration of 102.5 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 924800 dpm.

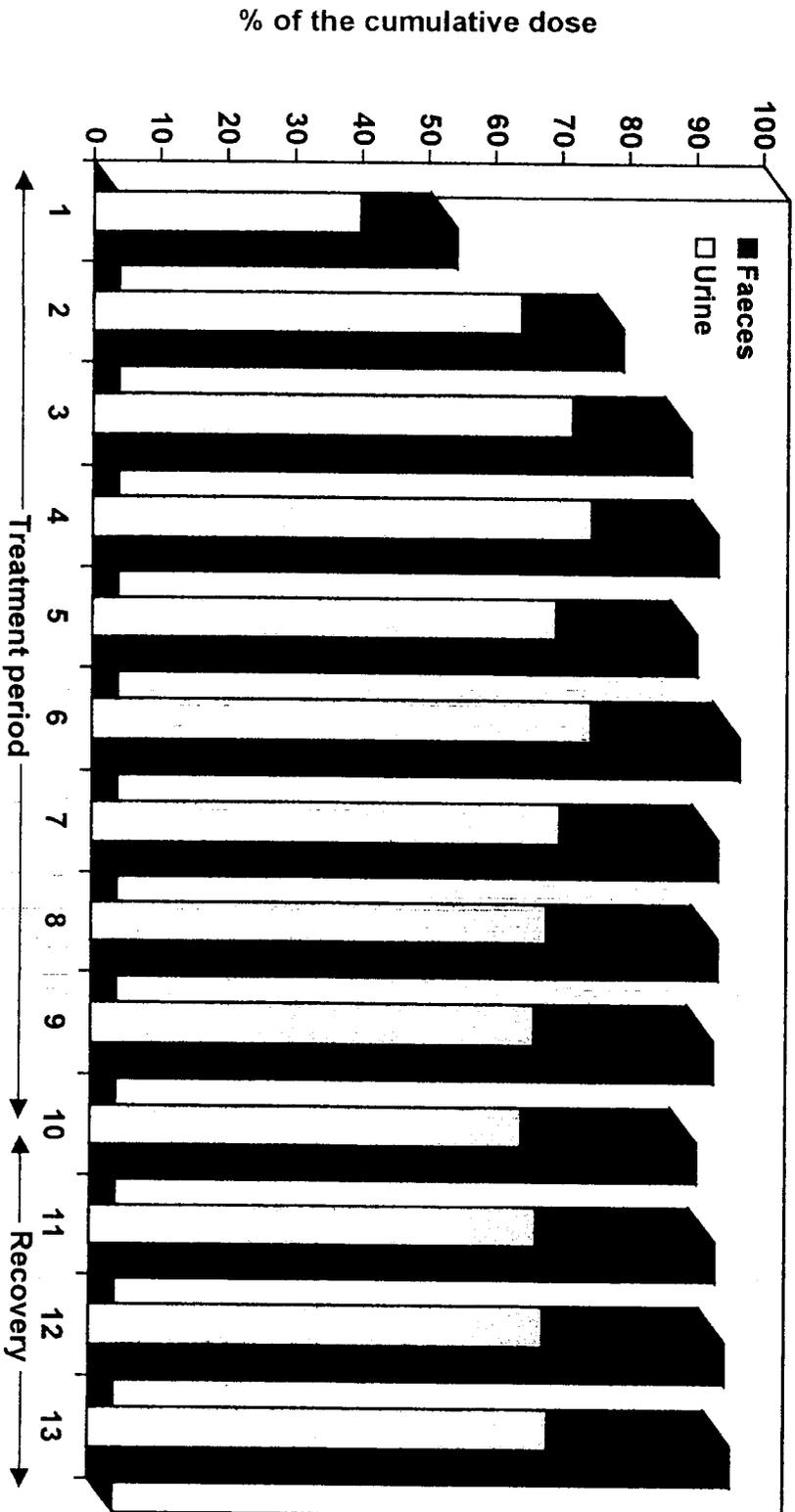


Figure 11. Mean recovery of radioactivity (in $\mu\text{mole DEHP-equivalents}$) in urine and faeces from female rats collected within 24 hours following the 1st to the 10th oral administration of 1000 mg/kg/d [^{14}C]-DEHP.

Rats received a 10-day repeated oral administration of 512.5 $\mu\text{moles/animal/d}$ of [^{14}C]-DEHP with a specific activity of 924800 dpm.

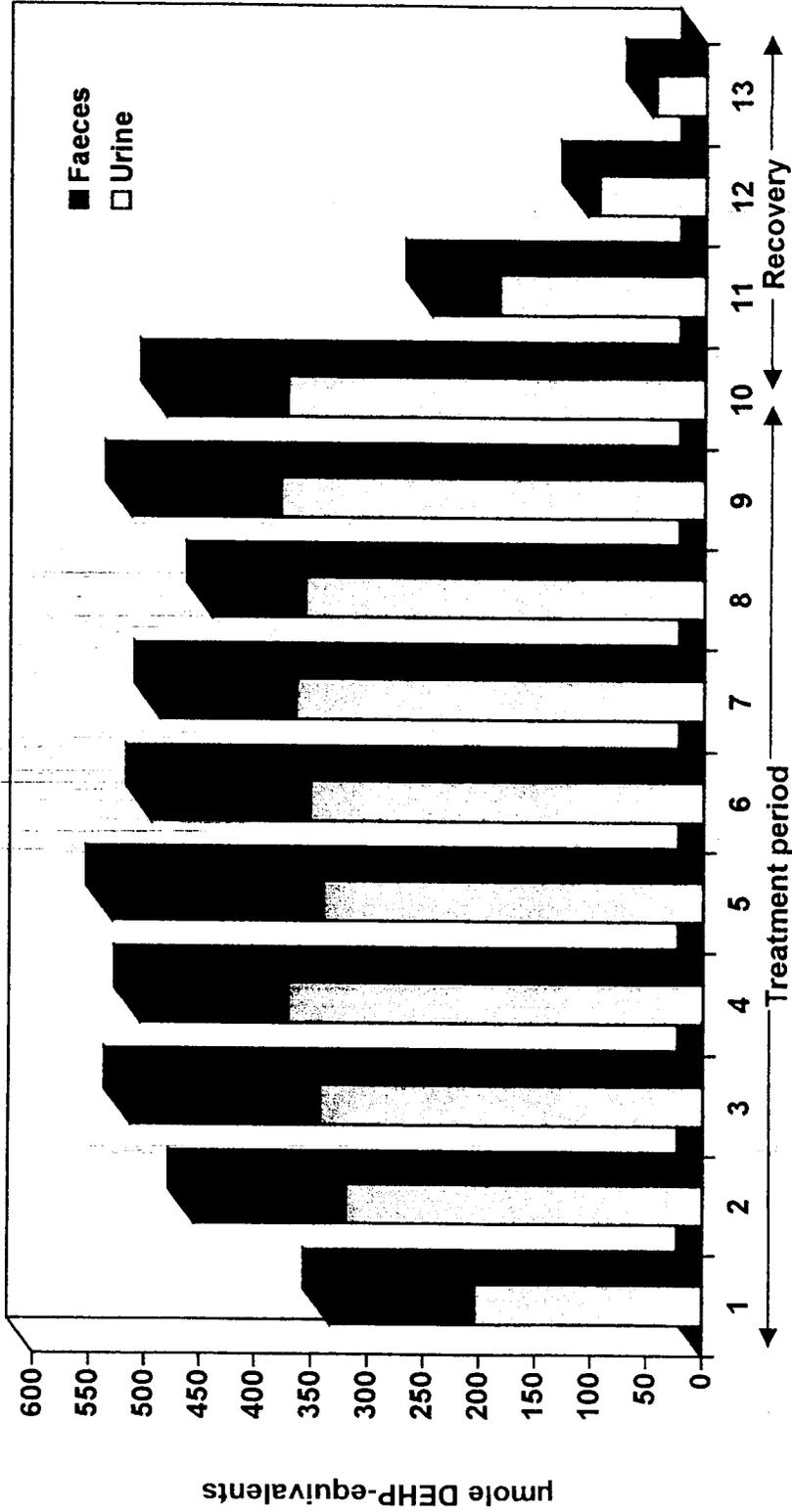


Figure 12. Mean recovery of radioactivity (in % of the cumulative dose) in urine and faeces from female rats collected within 24 hours following the 1st to the 10th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Rats received a 10-day repeated oral administration of 512.5 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 924800 dpm.

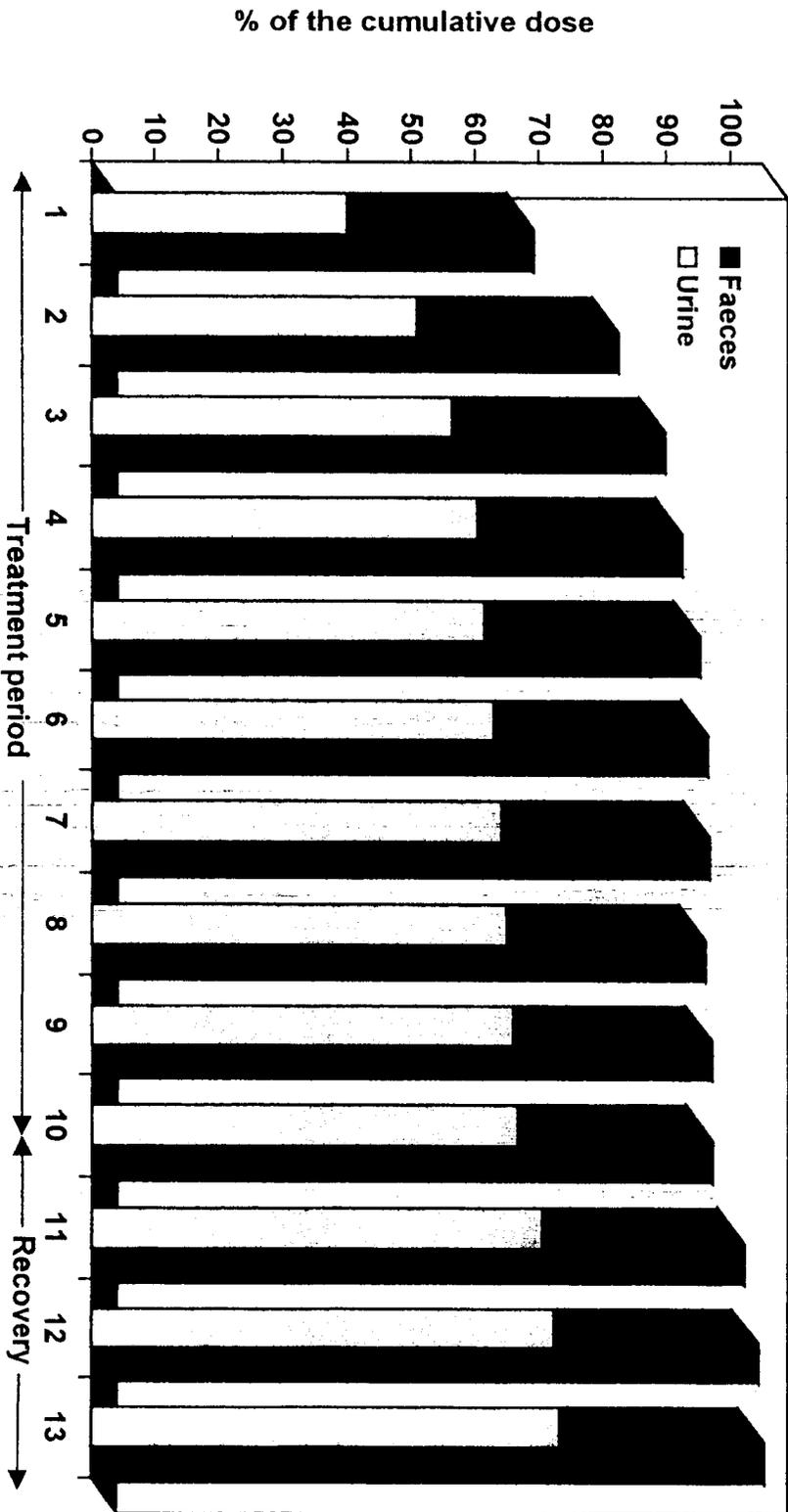


Figure 13. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female rats within 24 hours following the 1st, 4th, 7th and 10th oral administration of 200 mg/kg [¹⁴C]-DEHP.

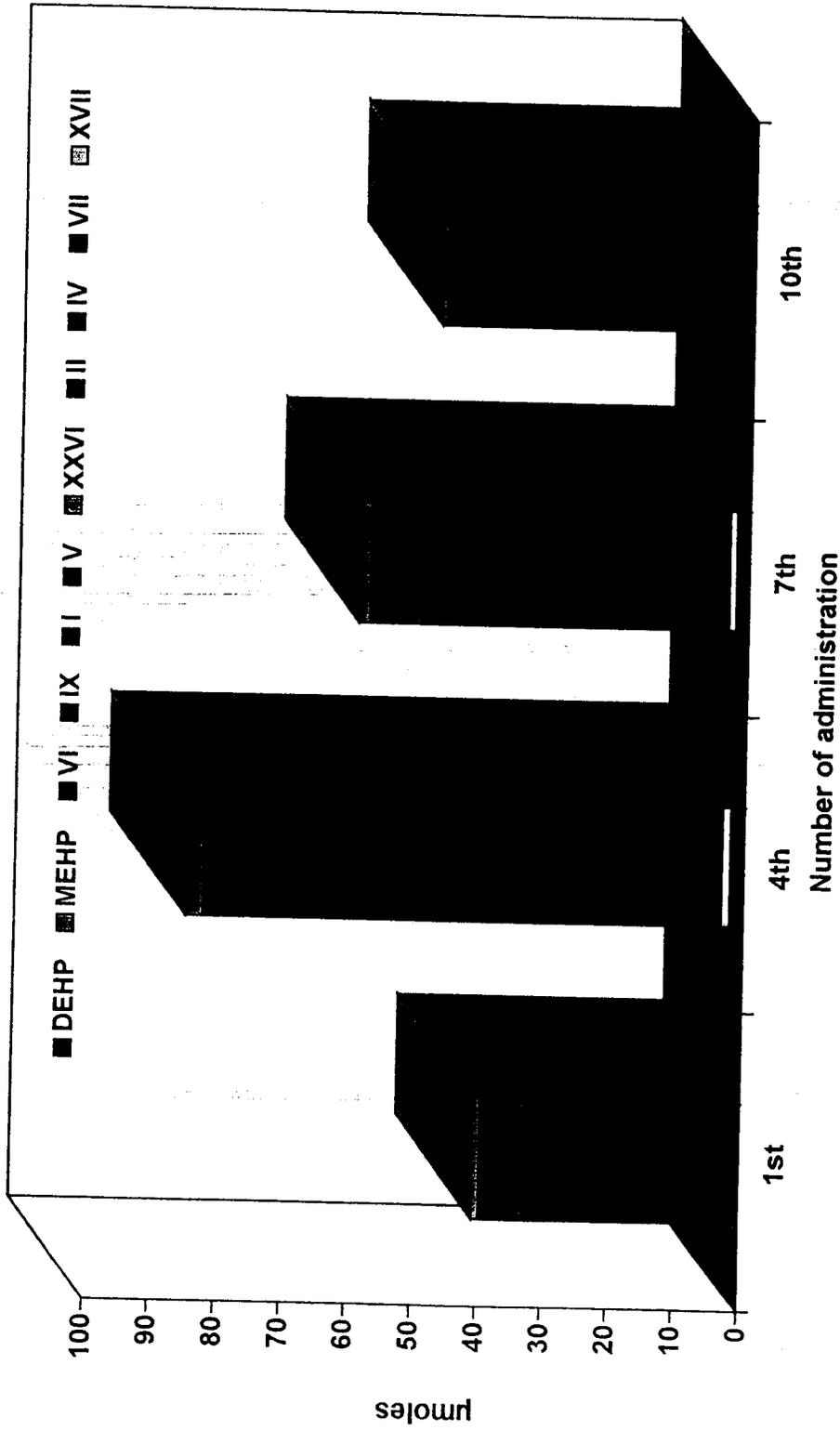


Figure 14. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in faeces from female rats within 24h hours following the 1st, 4th, 7th and 10th oral administration of 200 mg/kg [¹⁴C]-DEHP.

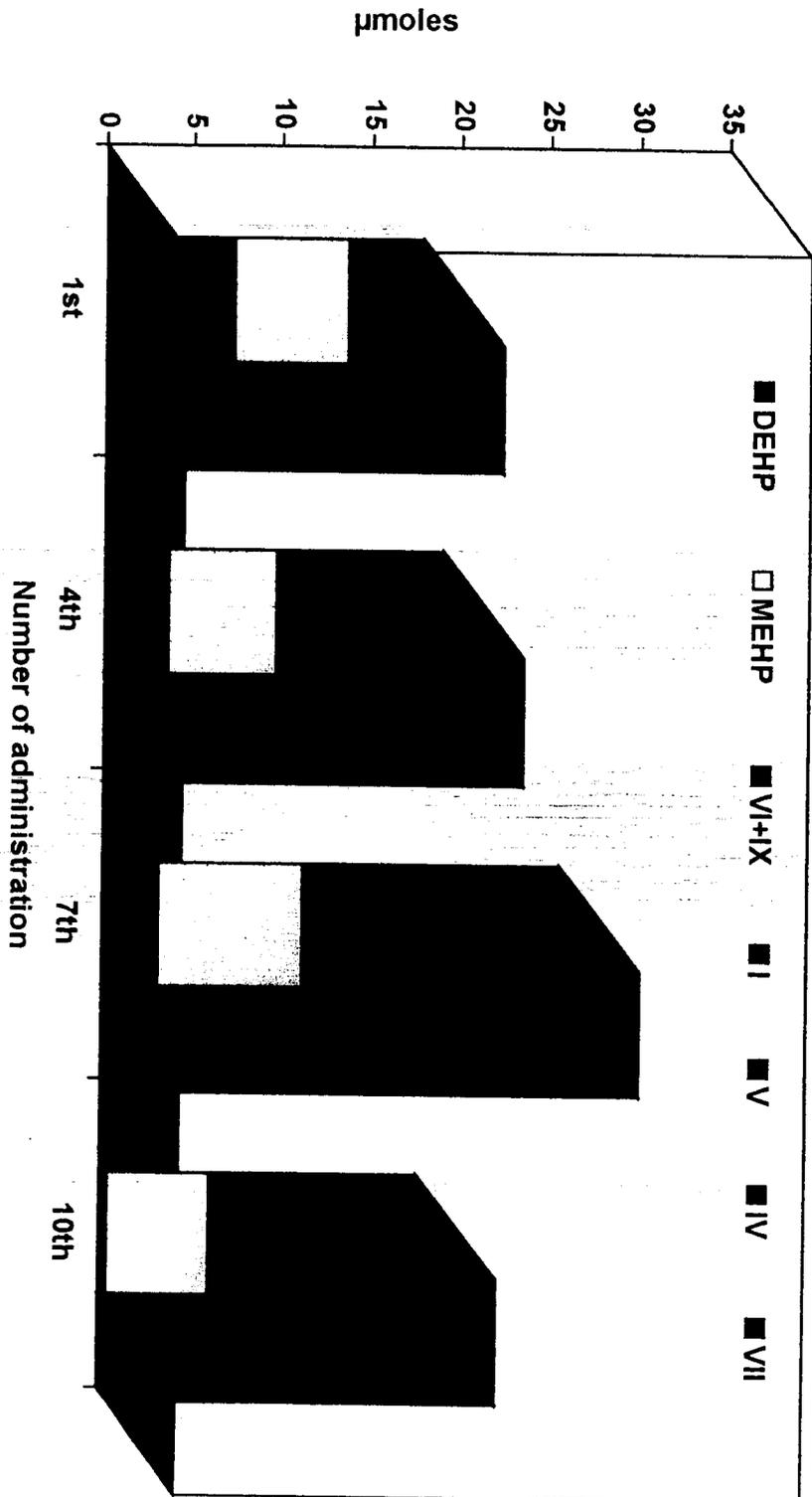


Figure 15. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in the urine and faeces of female rats within 24 hours following the 1st, 4th, 7th and 10th oral administration of 200 mg/kg [¹⁴C]-DEHP.

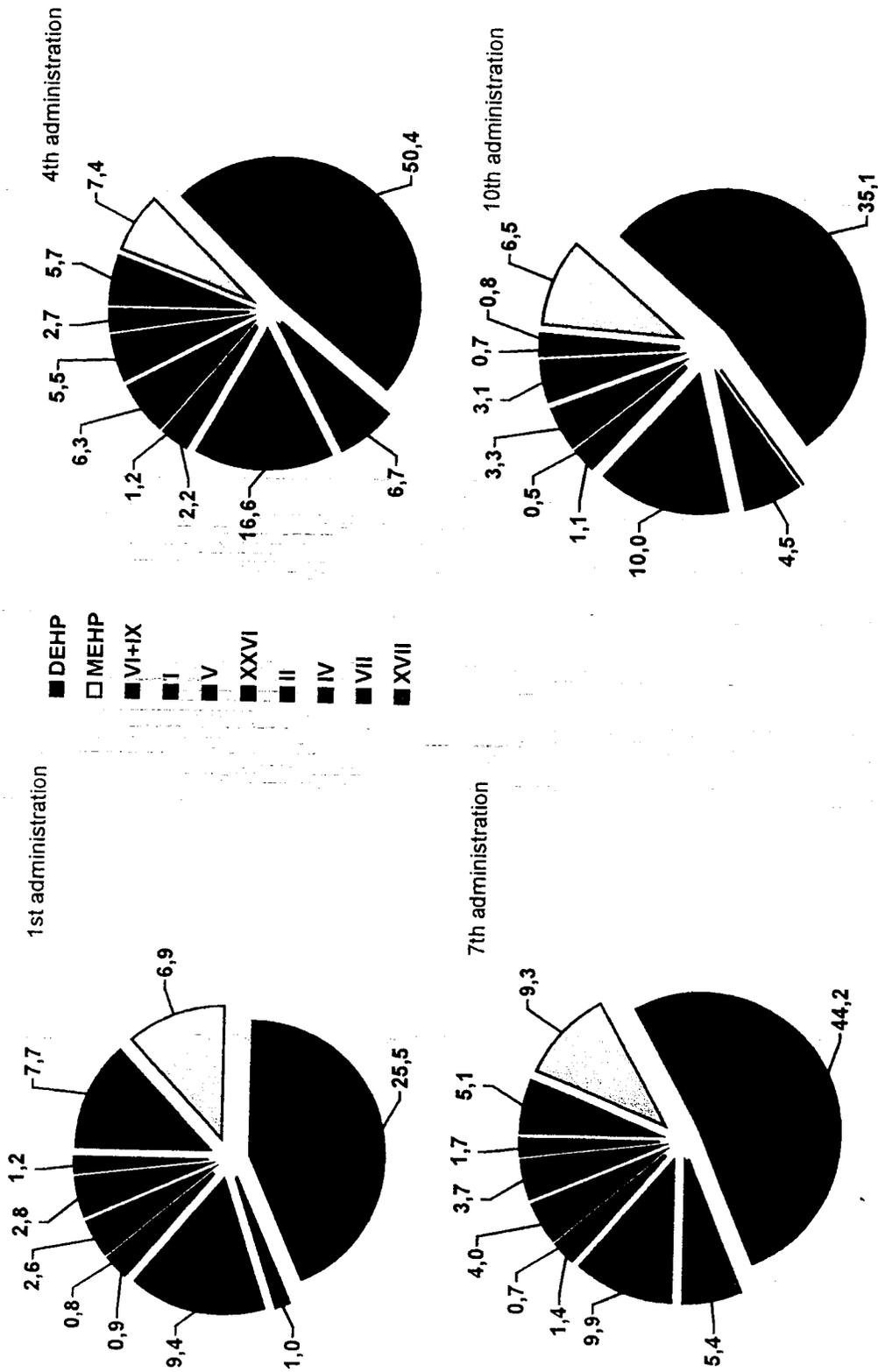


Figure 16. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in urine from female rats within 24 hours following the 1st, 4th, 7th and 10th oral administration of 1000 mg/kg [¹⁴C]-DEHP.

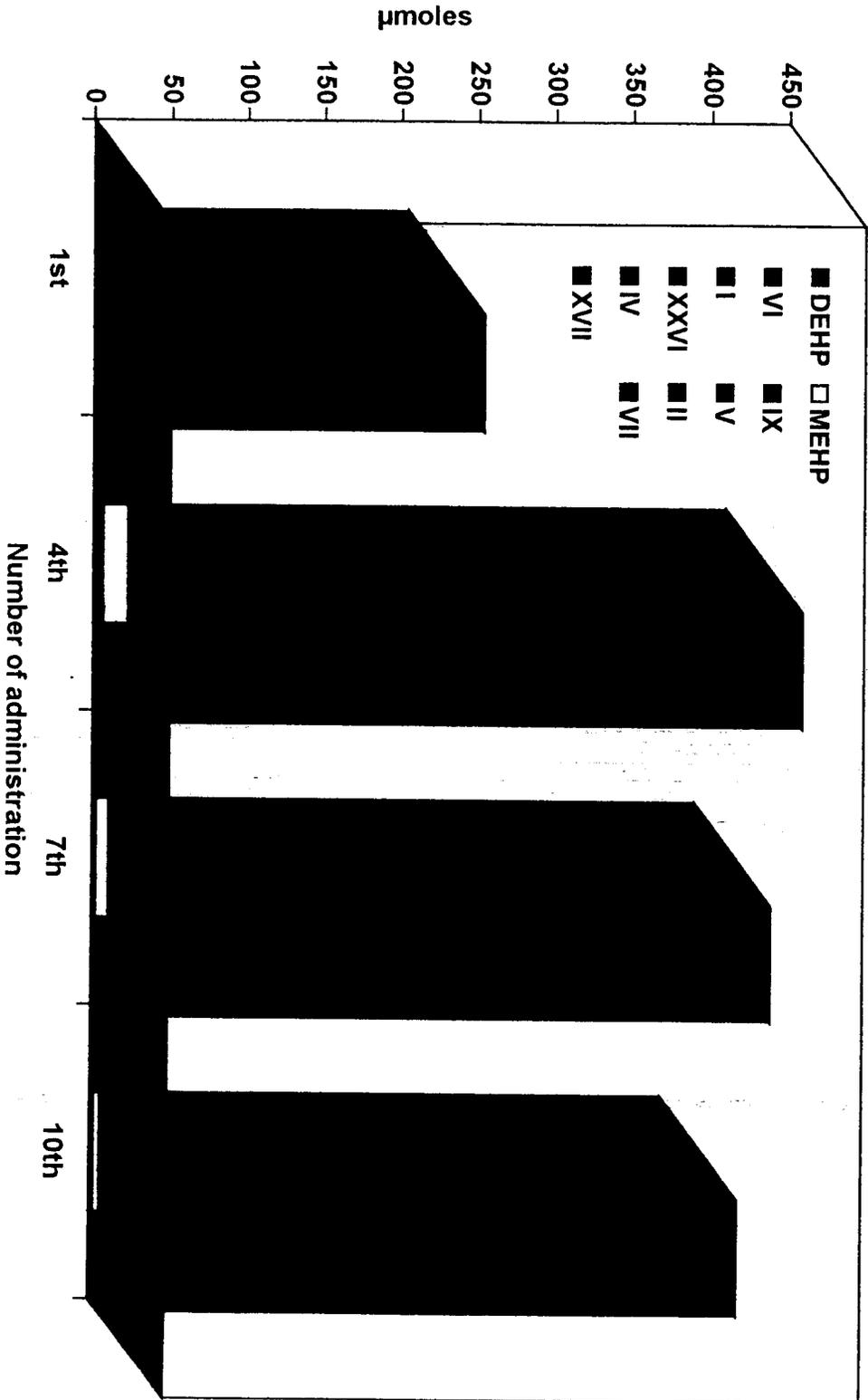


Figure 17. Recovery of DEHP, MEHP and MEHP-derived metabolites (in μmoles) in faeces from female rats within 24 hours following the 1st, 4th, 7th and 10th oral administration of 1000 mg/kg [¹⁴C]-DEHP.

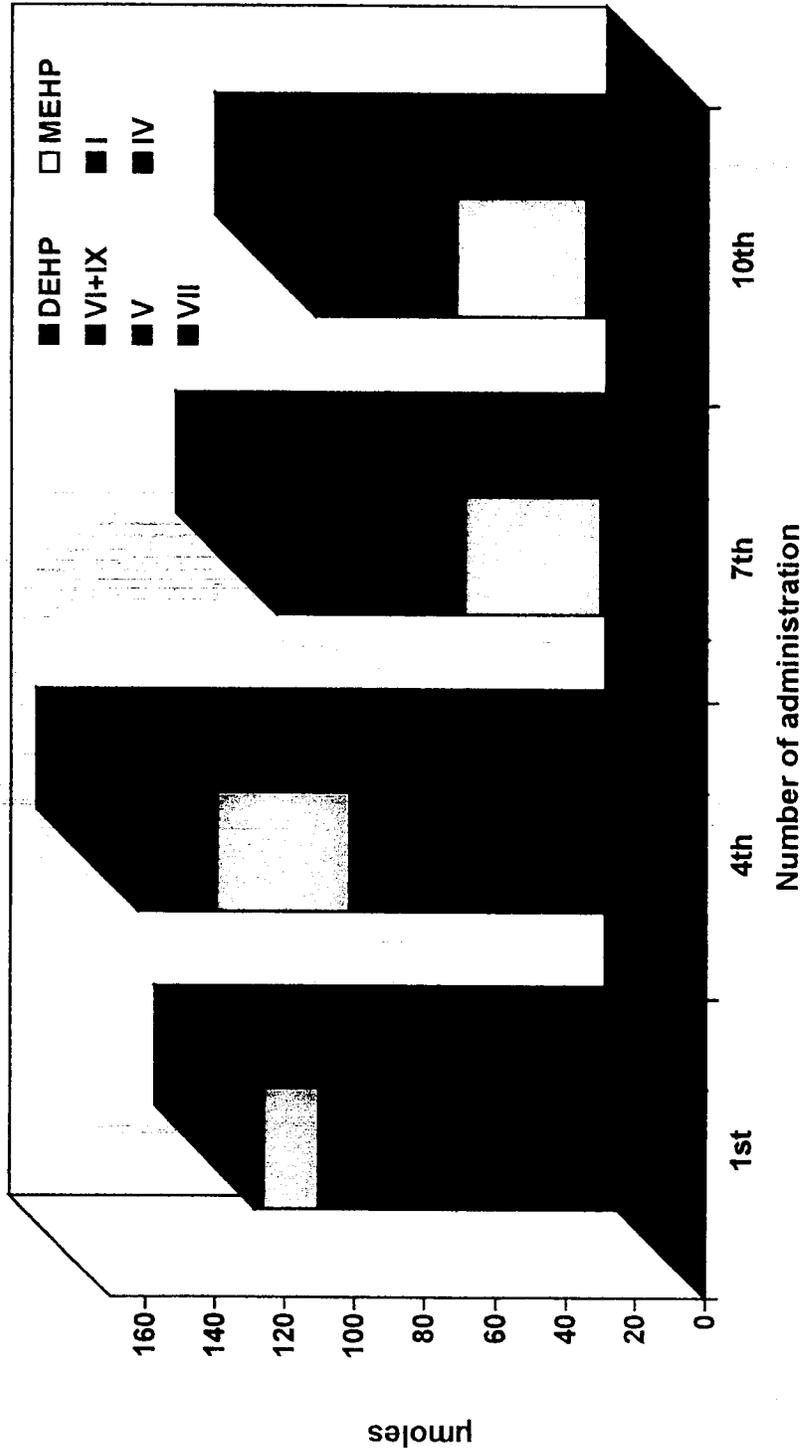
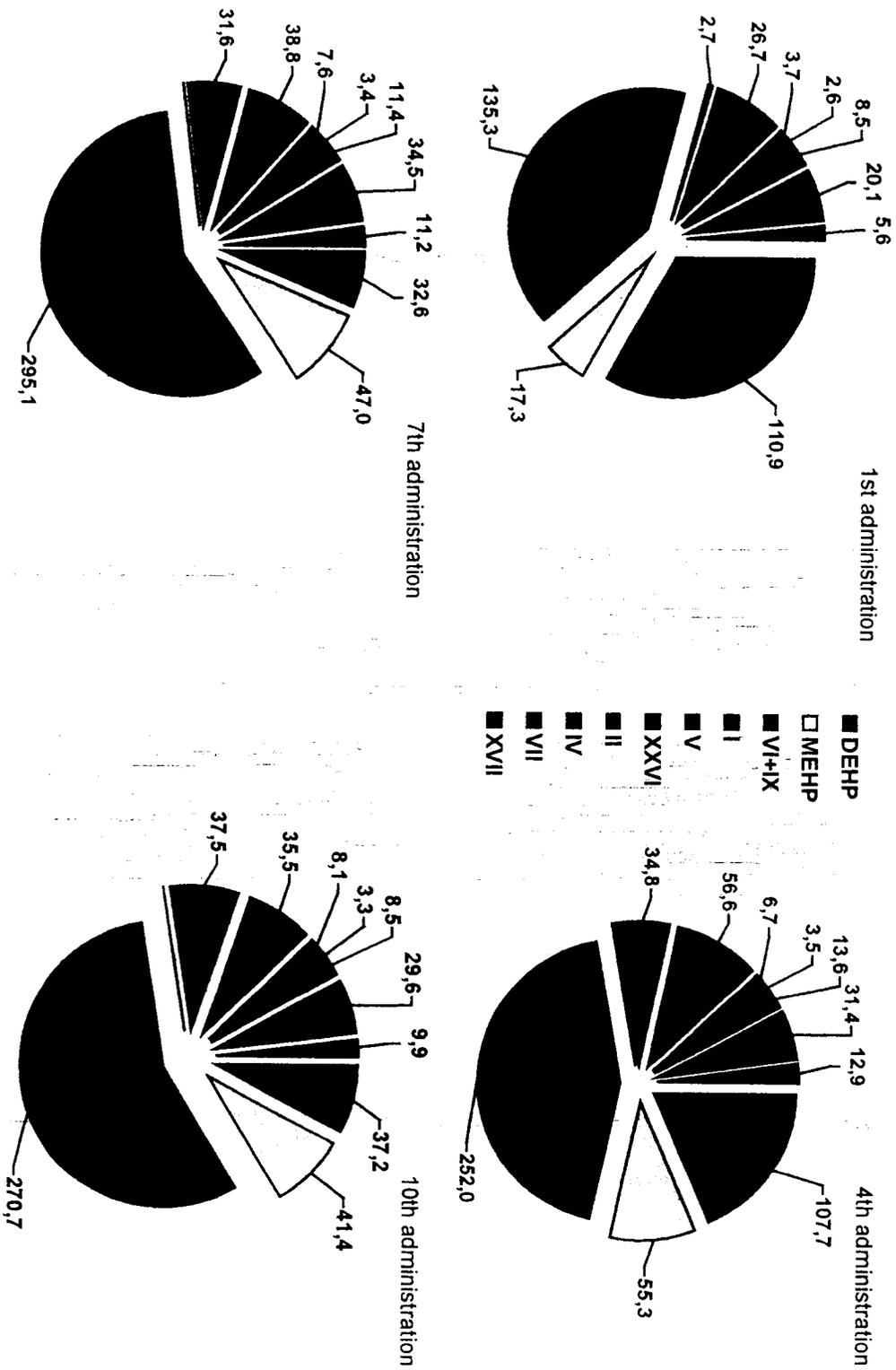


Figure 18. Cumulative excretion of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in the urine and faeces of female rats within 24 hours following the 1st, 4th, 7th and 10th oral administration of 1000 mg/kg [¹⁴C]-DEHP.



TABLES

Pharmacokinetic study

Table 1. Mean (and standard deviation) body weight values in female rats treated with a single administration of 200 or 1000 mg/kg DEHP on Day 0.

Day	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
D-3	194.1	5.9	6	191.9	4.2	6
D0	226.2	4.7	6	224.3	3.6	6
D1	227.7	4.8	6	225.3	6.0	6
D2	230.0	7.3	6	226.6	5.3	6
D3	230.9	6.9	6	226.5	8.2	6

Table 2. Concentration of radioactivity in blood (in nmole-DEHP-equivalents/g of blood) from female rats following a single oral administration of 200 or 1000 mg/kg [¹⁴C]-DEHP.

time after dosing	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
0.5h	42	9	3	226	56	3
1h	63	2	3	231	66	3
1h30	64	22	3	288	101	3
4h	46	14	3	353	101	3
24h	34	15	2	45	11	3
48h	0	0	2	17	11	3

Table 3. Mean (and standard deviation) body weight values in female rats treated with a 6-day repeated oral administration of 200 or 1000 mg/kg DEHP from D0 to D5 (inclusive).

Day	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
Reception	194.7	3.8	15	192.0	4.0	15
D-1	218.3	5.0	15	214.5	4.7	15
D0	217.8	5.2	15	215.8	5.5	15
D1	218.2	4.7	15	216.8	7.0	15
D2	220.5	4.2	15	218.3	5.8	15
D3	224.9	3.7	15	213.9	7.8	15
D4	226.0	5.9	15	213.0	8.3	15
D5	226.2	6.4	15	213.4	8.2	15
D6	226.7	5.7	15	216.8	7.5	15
D7	230.0	5.4	15	219.9	7.5	15
D8	231.8	9.9	15	217.7	9.2	15

Table 4. Concentration of radioactivity in blood (in nmole DEHP-equivalents/g of blood) from female rats after a single administration 200 or 1000 mg/kg of [¹⁴C]-DEHP which was preceded by a 5-day treatment with 200 or 1000 mg/kg/d unlabelled DEHP, respectively.

time after dosing	200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n
0.5h	24	14	3	271	59	3
1h	54	33	3	363	80	3
1h30	77	37	2	405	121	3
4h	60	15	3	342	55	3
24h	7	2	3	51	2	3
48h	2	1	3	42	32	3

Excretion and metabolism study, single dosing**Table 5. Mean (and standard deviation) body weight values in control female rats and in female rats treated with a single administration of 200 or 1000 mg/kg [¹⁴C]-DEHP on Day 0.**

Day	Control			200 mg/kg			1000 mg/kg		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
D-1	209.6	3.8	3	219.3	8.9	3	219.0	3.4	3
D0	212.3	5.1	3	221.3	9.3	3	218.6	6.6	3
D1	216.0	3.6	3	218.6	8.1	3	219.6	4.5	3
D2	218.0	2.6	3	219.6	7.8	3	224.3	6.0	3
D3	220.3	3.8	3	225.3	8.0	3	223.3	10.7	3
D4	225.0	4.6	3	227.0	9.8	3	225.3	8.9	3

Table 6. Recovery of radioactivity (as percentage of the [¹⁴C]-DEHP dose and as μmole DEHP-equivalents) in urine and faeces from female rats following a single oral administration of 200 mg/kg.

Interval	% of the dose						μmole DEHP-equivalents									
	Urine			Faeces			Total		Urine			Faeces			Total	
	Mean	SD	n	Mean	SD	n	Mean	SD	Mean	SD	n	Mean	SD	n	Mean	SD
0-24 h	48.9	14.5	3	26.8	5.3	3	75.7	19.8	50.2	14.9	3	27.5	5.5	3	77.6	20.4
24-48 h	5.4	0.7	3	5.0	0.7	2	10.4	1.4	5.6	0.8	3	5.1	0.7	2	10.7	1.5
48-72 h	2.3	2.0	3	0.8	1.3	3	3.1	3.3	2.3	2.1	3	0.9	1.3	3	3.2	3.4
72-96 h	1.0	0.4	3	0.3	0.2	3	1.3	0.6	1.0	0.4	3	0.3	0.2	3	1.3	0.6
Total	57.6	17.0	3	31.3	6.7	3	88.9	23.7	59.0	17.4	3	32.1	6.8	3	91.1	24.2

Table 7. Recovery of radioactivity (in % of the [¹⁴C]-DEHP dose and μmole DEHP-equivalents) in urine and faeces from female rats following a single oral administration of 1000 mg/kg.

Interval	% of the dose						μmole DEHP-equivalents									
	Urine			Faeces			Total		Urine			Faeces			Total	
	Mean	SD	n	Mean	SD	n	Mean	SD	Mean	SD	n	Mean	SD	n	Mean	SD
0-24 h	32.8	5.2	3	26.2	15.0	3	59.0	20.2	167.9	26.4	3	134.3	76.7	3	302.2	103.1
24-48 h	9.9	7.9	3	12.8	3.0	3	22.7	10.9	50.9	40.2	3	65.6	15.4	3	116.5	55.6
48-72 h	3.5	4.3	3	1.9	1.8	3	5.4	6.1	18.0	21.9	3	9.6	9.0	3	27.6	30.9
72-96 h	1.1	1.4	3	0.3	0.2	3	1.5	1.6	5.9	7.3	3	1.7	1.1	3	7.6	8.4
Total	47.4	12.6	3	41.2	15.4	3	88.6	6.9	242.7	64.7	3	211.2	78.7	3	453.9	143.4

Table 8. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in urine from female rats following a single oral administration of 200 mg/kg.

Metabolites	μ moles												% of the dose											
	0-24 h			24-48 h			48-72 h			Total			0-24 h			24-48 h			48-72 h			Total		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	0.72	0.42	3	0.57	0.18	3	0.55	0.60	3	1.84	1.20		0.70	0.41	3	0.56	0.18	3	0.54	0.58	3	1.80	1.17	
I	1.65	0.33	3	0.23	0.06	3	0.08	0.08	3	1.96	0.47		1.61	0.32	3	0.23	0.06	3	0.08	0.08	3	1.92	0.46	
II	0.47	0.13	3	0.03	0.01	3	0.00	0.00	3	0.50	0.14		0.46	0.13	3	0.03	0.01	3	0.00	0.00	3	0.49	0.14	
IV	2.50	0.84	3	0.29	0.12	3	0.11	0.11	3	2.90	1.07		2.44	0.82	3	0.29	0.12	3	0.10	0.10	3	2.83	1.04	
V	9.98	2.99	3	0.53	0.21	3	0.22	0.19	3	10.73	3.39		9.74	2.92	3	0.52	0.20	3	0.22	0.18	3	10.48	3.30	
VI	10.11	2.61	3	1.15	0.38	3	0.44	0.35	3	11.7	3.34		9.86	2.54	3	1.12	0.37	3	0.43	0.34	3	11.41	3.25	
VII	3.51	1.56	3	0.19	0.06	3	0.14	0.08	3	3.84	1.70		3.43	1.52	3	0.19	0.06	3	0.14	0.07	3	3.76	1.65	
IX	18.60	5.88	3	1.23	0.41	3	0.52	0.50	3	20.35	6.79		18.14	5.74	3	1.20	0.40	3	0.51	0.48	3	19.85	6.62	
XVII	1.11	0.36	3	0.22	0.06	3	0.11	0.11	3	1.44	0.53		1.08	0.36	3	0.22	0.05	3	0.10	0.10	3	1.40	0.51	
XXVI	0.94	0.23	3	0.16	0.04	3	0.05	0.07	3	1.15	0.34		0.92	0.22	3	0.16	0.04	3	0.05	0.07	3	1.13	0.33	
DEHP	0.55	0.10	3	0.92	0.63	3	0.10	0.08	3	1.57	0.81		0.54	0.09	3	0.90	0.61	3	0.10	0.08	3	1.54	0.78	
Total	50.1	14.8	3	5.5	0.8	3	2.3	2.1	3	57.9	17.7		48.9	14.5	3	5.4	0.7	3	2.2	2.0	3	56.5	17.2	

Table 9. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in faeces from female rats following a single oral administration of 200 mg/kg.

Metabolites	μ moles										% of the dose																						
	0-24 h			24-48 h			48-72 h			Total			0-24 h			24-48 h			48-72 h			Total											
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n									
MEHP	8.33	2.25	3	1.58	0.17	2	0.29	-	1	10.20	2.42	8.12	2.20	3	1.54	0.12	2	0.28	-	1	9.94	2.32	1.64	0.37	3	0.67	0.02	2	1.03	-	1	3.34	0.39
V	1.68	0.38	3	0.69	0.03	2	1.06	-	1	3.43	0.41	1.86	1.28	3	2.17	0.70	2	0.94	-	1	4.97	1.98	15.19	5.06	3	0.57	0.07	2	0.03	0.04	3	15.79	5.17
VI+IX	1.90	1.31	3	2.23	1.02	2	0.96	-	1	5.09	2.33	26.8	8.9	3	5.0	0.9	2	2.3	0.04	34.1	9.86	DEHP	15.57	5.19	3	0.59	0.11	2	0.03	0.05	3	16.19	5.35
Total	27.5	9.1	3	5.1	1.3	2	2.3	0.05	34.9	10.5	Total	26.8	8.9	3	5.0	0.9	2	2.3	0.04	34.1	9.86												

Table 10. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in urine from female rats following a single oral administration of 1000 mg/kg.

Metabolites	μ moles												% of the dose											
	0-24 h			24-48 h			48-72 h			Total			0-24 h			24-48 h			48-72 h			Total		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	0.94	0.11	3	0.90	0.23	3	0.99	0.63	3	2.83	0.97		0.18	0.02	3	0.17	0.04	3	0.19	0.12	3	0.54	0.18	
I	6.25	3.03	3	3.00	2.49	3	0.87	1.08	3	10.12	6.60		1.22	0.59	3	0.59	0.49	3	0.17	0.21	3	1.98	1.29	
II	1.28	0.11	3	0.35	0.29	3	0.14	0.18	3	1.77	0.58		0.25	0.02	3	0.07	0.06	3	0.03	0.03	3	0.35	0.11	
IV	6.93	2.97	3	2.20	1.13	3	0.72	0.77	3	9.85	4.87		1.35	0.58	3	0.43	0.22	3	0.14	0.15	3	1.92	0.95	
V	34.97	5.27	3	8.08	7.47	3	2.80	3.86	3	45.85	16.60		6.82	1.03	3	1.58	1.46	3	0.55	0.75	3	8.95	3.24	
VI	39.39	7.69	3	13.85	11.30	3	4.72	6.07	3	57.96	25.06		7.69	1.50	3	2.70	2.20	3	0.92	1.18	3	11.31	4.88	
VII	15.23	2.40	3	4.00	3.84	3	1.35	1.86	3	20.58	8.10		2.97	0.47	3	0.78	0.75	3	0.26	0.36	3	4.01	1.58	
IX	56.08	7.31	3	15.05	12.02	3	5.20	6.51	3	76.33	25.84		10.94	1.43	3	2.94	2.35	3	1.01	1.27	3	14.89	5.05	
XVII	3.62	0.69	3	1.26	0.82	3	0.38	0.28	3	5.26	1.79		0.71	0.14	3	0.25	0.16	3	0.07	0.05	3	1.03	0.35	
XXVI	2.15	0.75	3	1.10	0.71	3	0.41	0.43	3	3.66	1.89		0.42	0.15	3	0.21	0.14	3	0.08	0.08	3	0.71	0.37	
DEHP	1.10	0.32	3	1.13	0.58	3	0.40	0.30	3	2.63	1.20		0.22	0.06	3	0.22	0.11	3	0.08	0.06	3	0.52	0.23	
Total	168.0	26.4	3	50.9	40.3	3	18.0	21.9	3	236.9	88.6		32.8	5.2	3	9.9	7.9	3	3.5	4.3	3	46.2	17.4	

Table 11. Mean quantity of DEHP, MEHP and MEHP-derived metabolites (in μ moles and % of the DEHP dose) excreted in faeces from female rats following a single oral administration of 1000 mg/kg.

Metabolites	μ moles									% of the dose														
	0-24 h			24-48 h			48-72 h			Total			0-24 h			24-48 h			48-72 h			Total		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	15.61	11.26	3	16.05	9.71	3	1.14	0.65	3	32.80	21.60	3	3.04	1.79	3	3.13	1.54	3	0.22	0.13	3	6.39	3.5	3
V	3.18	1.52	3	6.47	3.69	3	2.66	2.00	3	12.31	7.21	3	0.62	0.24	3	1.26	0.72	3	0.52	0.39	3	2.40	1.3	3
VI+IX	4.10	2.81	3	2.95	1.46	3	5.67	4.74	3	12.72	9.01	3	0.80	0.44	3	0.76	0.15	3	1.10	0.93	3	2.66	1.5	3
DEHP	111.4	51.32	3	40.10	20.83	3	0.16	0.11	3	151.66	72.26	3	21.73	10.01	3	7.82	4.06	3	0.03	0.02	3	29.58	14.1	3
Total	134.3	66.9	3	65.6	35.7	3	9.6	7.5	3	209.5	110.1	3	26.2	12.5	3	12.8	6.5	3	1.9	1.5	3	41.0	20.4	3

Excretion and metabolism study, repeated dosing

Table 12. Mean (and standard deviation) body weight values in control female rats and in female rats treated with a 10-day repeated oral administration of 200 or 1000 mg/kg [¹⁴C]-DEHP.

Day	Control		200 mg/kg			1000 mg/kg		
	Mean	n	Mean	SD	n	Mean	SD	n
D-1	236	1	236.2	3.8	5	234.8	5.3	5
D0	227	1	233.2	4.5	5	237.6	2.5	5
D1	232	1	225.0	10.3	5	238.8	2.8	5
D2	237	1	228.8	15.1	5	233.0	3.4	5
D3	239	1	228.6	18.4	5	228.8	5.5	5
D4	233	1	228.0	17.1	5	232.8	5.2	5
D5	234	1	233.4	12.5	5	237.4	4.1	5
D6	237	1	235.4	10.7	5	242.2	5.4	5
D7	237	1	240.0	10.3	4	242.6	2.9	5
D8	235	1	245.8	8.9	4	238.4	6.7	5
D9	238	1	247.8	8.4	4	242.0	4.9	5
D10	242	1	248.5	15.3	4	243.6	4.5	5
D11	237	1	253.8	10.0	4	248.0	5.4	5

Table 13. Mean recovery of radioactivity in urine from female rats collected within 24 hours following the 1st to the 10th oral administration of 200 mg/kg/d [¹⁴C]-DEHP.

Rats received a 10-day repeated oral administration of 102.5 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 924800 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	40.8	23.0	5	39.8	22.4	5	40.8	23.0	5	39.8	22.4	5
R	2	90.4	14.7	5	88.2	14.3	5	131.2	20.1	5	64.0	9.8	5
E	3	89.2	51.0	5	87.1	49.8	5	220.4	58.3	5	71.7	18.9	5
A	4	85.4	36.0	5	83.4	35.1	5	305.8	82.2	5	74.6	20.1	5
T	5	49.4	30.2	5	48.2	29.4	5	355.3	99.0	5	69.3	19.3	5
M	6	103.3	18.5	5	100.8	18.0	5	458.6	101.1	5	74.6	16.4	5
E	7	59.7	35.1	4	58.2	34.2	4	502.5	134.2	4	70.0	18.7	4
N	8	56.2	26.7	4	54.9	26.0	4	558.7	155.7	4	68.1	19.0	4
T	9	53.3	31.5	4	52.0	30.7	4	612.0	185.6	4	66.3	20.1	4
P	10	47.7	22.2	4	46.5	21.7	4	659.7	193.4	4	64.4	18.9	4
E	11	31.8	16.5	3	-	-	-	683.5	213.6	4	66.7	20.8	4
R	12	17.3	6.7	3	-	-	-	696.5	221.6	4	67.9	21.6	4
I	13	8.0	2.5	4	-	-	-	704.5	219.8	4	68.7	21.4	4

Table 14. Mean recovery of radioactivity in faeces from female rats collected within 24 hours following the 1st to the 10th oral administration of 200 mg/kg/d [¹⁴C]-DEHP.

Rats received a 10-day repeated oral administration of 102.5 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 924800 dpm.

Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	10.8	9.9	5	10.5	9.6	5	10.8	9.9	5	10.5	9.6	5
R	12.4	11.4	5	12.1	11.2	5	23.2	21.3	5	11.3	10.4	5
E	18.9	17.9	5	18.4	17.5	5	42.0	37.4	5	13.7	12.2	5
A	19.2	5.9	5	18.7	5.8	5	61.3	37.8	5	14.9	9.2	5
T	26.6	10.9	5	25.9	10.6	5	87.8	46.0	5	17.1	9.0	5
M	23.9	16.5	5	23.4	16.1	5	111.8	39.7	5	18.2	6.4	5
E	25.7	3.0	4	25.1	3.0	4	141.5	45.1	4	19.7	6.3	4
N	35.8	6.6	4	35.0	6.4	4	177.3	44.2	4	21.6	5.4	4
T	32.9	12.6	4	32.1	12.2	4	210.2	51.3	4	22.8	5.6	4
P	17.9	7.8	4	17.5	7.6	4	228.1	53.8	4	22.3	5.2	4
E	5.9	1.6	4	-	-	-	234.0	54.5	4	22.8	5.3	4
R	2.9	1.0	4	-	-	-	236.9	55.4	4	23.1	5.4	4
I	1.1	0.4	4	-	-	-	237.9	55.6	4	23.2	5.4	4
O												
D												
RE												
CO												
VE												
RY												

Table 15. Mean recovery of radioactivity in urine from female rats collected within 24 hours following the 1st to the 10th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Rats received a 10-day repeated oral administration of 512.5 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 924800 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	204.2	28.4	4	39.9	5.5	4	204.2	28.4	4	39.9	5.5	4
R	2	319.3	100.0	5	62.3	19.5	5	482.7	168.8	5	51.0	8.8	5
E	3	343.5	86.5	5	67.0	16.9	5	826.2	249.7	5	56.5	11.0	5
A	4	371.6	88.7	5	72.5	17.3	5	1197.8	333.9	5	60.5	12.0	5
T	5	341.4	156.9	5	66.6	30.6	5	1539.2	474.5	5	61.5	15.5	5
M	6	353.1	128.9	5	68.9	25.1	5	1892.2	529.1	5	63.0	14.6	5
E	7	366.6	149.7	5	71.5	29.2	5	2258.8	523.7	5	64.2	11.9	5
N	8	357.7	95.0	5	69.8	18.5	5	2616.5	535.0	5	65.0	10.6	5
T	9	380.2	120.9	5	74.2	23.6	5	2996.7	593.2	5	66.1	10.8	5
P	10	374.2	155.1	5	73.0	30.3	5	3370.9	542.1	5	66.8	8.4	5
E	11	184.8	72.2	5	-	-	-	3555.7	492.4	5	70.6	7.2	5
R	12	96.0	45.0	5	-	-	-	3651.7	497.5	5	72.5	7.2	5
I	13	45.3	31.3	5	-	-	-	3697.0	499.4	5	73.4	7.1	5

Table 16. Mean recovery of radioactivity in faeces from female rats collected within 24 hours following the 1st to the 10th oral administration of 1000 mg/kg/d [¹⁴C]-DEHP.

Rats received a 10-day repeated oral administration of 512.5 µmoles/animal/d of [¹⁴C]-DEHP with a specific activity of 924800 dpm.

	Days	Daily excretion in µmole DEHP-equivalents			Daily excretion in % of the daily dose			Cumulative excretion in µmole DEHP-equivalents			Cumulative excretion in % of the cumulative dose		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
T	1	129.0	73.0	4	25.2	14.2	4	129.0	73.0	4	25.2	14.2	4
R	2	136.2	77.0	5	26.6	15.0	5	239.4	42.8	5	27.5	8.9	5
E	3	170.4	138.0	5	33.2	26.9	5	409.8	173.8	5	29.3	12.6	5
A	4	134.2	123.7	5	26.2	24.1	5	544.0	179.8	5	27.9	8.1	5
T	5	189.9	108.1	5	37.1	21.1	5	733.9	231.6	5	29.7	8.3	5
M	6	143.2	101.3	5	28.0	19.8	5	877.2	281.5	5	29.4	8.5	5
E	7	121.8	9.3	5	23.8	1.8	5	999.0	276.5	5	28.5	7.1	5
N	8	83.7	65.8	5	16.3	12.9	5	1082.7	305.3	5	27.0	6.9	5
T	9	134.9	45.1	5	26.3	8.8	5	1217.6	321.5	5	26.9	6.4	5
P	10	109.6	90.2	5	21.4	17.6	5	1327.2	392.3	5	26.3	7.3	5
R	11	60.6	17.5	5	-	-	-	1387.9	382.2	5	27.5	7.1	5
E	12	10.5	2.6	5	-	-	-	1398.4	381.9	5	27.8	7.1	5
O	13	3.5	1.3	5	-	-	-	1401.8	381.8	5	27.8	7.0	5

Table 17. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female rats collected within 24 hours following the 1st, 4th, 7th and 10th oral administration of 200 mg/kg/d.

Treatment	1 st			4 th			7 th			10 th		
Metabolites	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	0.47	0.18	5	1.34	0.16	5	1.18	0.83	4	0.65	0.50	4
I	0.98	0.73	5	6.44	2.73	5	4.24	1.78	4	3.78	0.39	4
II	0.76	0.86	5	1.20	0.40	5	0.74	0.93	4	0.51	0.24	4
IV	2.45	1.49	5	5.05	1.40	5	2.71	0.31	4	2.11	0.83	4
V	8.24	4.35	5	14.13	6.32	5	7.11	1.69	4	7.41	3.44	4
VI	8.09	4.86	5	22.69	10.93	5	17.48	12.53	4	14.45	8.52	4
VII	2.71	1.88	5	5.30	5.37	5	2.95	3.26	4	2.57	0.95	4
IX	14.52	8.57	5	22.36	8.60	5	18.22	14.90	4	13.99	6.57	4
XVII	1.16	0.61	5	2.68	1.19	5	1.73	0.84	4	0.73	0.23	4
XXVI	0.90	0.66	5	2.21	0.90	5	1.44	0.85	4	1.14	0.52	4
DEHP	0.46	0.56	5	2.04	1.06	5	1.87	0.94	4	0.33	0.36	4
Total	40.74	23.02	5	85.44	35.97	5	59.65	35.08	4	47.63	22.18	4

Table 18. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female rats collected within 24 hours following the 1st, 4th, 7th and 10th oral administration of 200 mg/kg/d.

Treatment	1 st			4 th			7 th			10 th		
Metabolites	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	6.42	0.82	3	6.10	2.61	5	8.14	2.98	4	5.84	2.13	4
I	0.00	0.00	3	0.21	0.29	5	1.12	0.44	4	0.74	0.60	4
IV	0.10	0.17	3	1.20	1.04	5	1.30	0.73	4	1.14	0.71	4
V	1.16	0.26	3	2.44	0.71	5	2.76	1.02	4	2.60	0.95	4
VII	0.09	0.15	3	0.19	0.32	5	0.71	0.04	4	0.50	0.30	4
VI+IX	2.85	0.28	3	5.32	2.01	5	8.45	1.87	4	6.64	3.30	4
DEHP	7.27	0.30	3	3.67	2.89	5	3.24	2.83	4	0.45	0.34	4
Total	17.90	1.15	3	19.14	5.92	5	25.73	3.00	4	17.90	7.80	4

Table 19. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in urine from female rats collected within 24 hours following the 1st, 4th, 7th and 10th oral administration of 1000 mg/kg/d.

Metabolites	1 st			4 th			7 th			10 th		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	1.54	1.71	4	16.87	3.91	4	8.20	2.59	4	4.34	2.49	4
I	2.67	2.40	4	33.49	11.56	4	27.75	14.74	4	33.28	12.12	4
II	2.56	1.26	4	3.49	0.56	4	3.43	2.19	4	3.30	1.81	4
IV	8.36	2.70	4	12.29	8.73	4	8.66	4.38	4	6.38	1.99	4
V	25.82	14.46	4	47.05	19.56	4	27.02	5.90	4	25.49	8.82	4
VI	61.63	17.67	4	144.30	25.80	4	159.18	90.57	4	154.11	89.17	4
VII	19.99	7.22	4	27.75	3.42	4	30.33	20.88	4	26.78	13.18	4
IX	72.33	14.72	4	99.27	9.64	4	104.67	54.36	4	95.59	40.87	4
XVII	5.37	1.57	4	12.87	0.53	4	11.20	6.25	4	9.88	7.98	4
XXVI	3.69	1.45	4	6.73	0.37	4	7.62	3.50	4	8.14	4.38	4
DEHP	0.28	0.31	4	5.86	3.39	4	2.30	1.46	4	2.36	1.42	4
Total	204.23	28.54	4	409.98	23.14	4	390.35	161.62	4	369.65	178.56	4

Table 20. Mean recovery of DEHP, MEHP and MEHP-derived metabolites (in μ moles) in faeces from female rats collected within 24 hours following the 1st, 4th, 7th and 10th oral administration of 1000 mg/kg/d.

Metabolites	1 st			4 th			7 th			10 th		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
MEHP	15.74	8.87	4	38.39	14.46	4	38.76	7.98	4	37.01	32.17	4
I	0.05	0.10	4	1.33	0.96	4	3.85	1.38	4	4.25	2.59	4
IV	0.18	0.35	4	1.32	1.10	4	2.78	1.76	4	2.16	1.56	4
V	0.91	0.14	4	9.55	6.09	4	11.82	3.79	4	10.03	6.96	4
VII	0.07	0.08	4	1.80	1.52	4	4.18	2.16	4	2.78	1.47	4
VI+IX	1.35	0.38	4	8.47	5.84	4	31.20	17.70	4	20.99	10.77	4
DEHP	110.61	64.75	4	101.84	117.97	4	30.54	13.50	4	34.85	50.86	4
Total	128.90	72.92	4	162.70	122.17	4	123.13	10.02	4	112.08	103.98	4