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May 28, 2003

Charlie Auer, Director
TSCA Document Control Office (7407)
EPA East Building, Room 6428
Office of Pollution Prevention and Toxics
U S Environmental Protection Agency
1201 Constitution Avenue, NW
Washington, DC 20460-0001



Dear Mr. Auer,

The American Chemistry Council makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, I am pleased to send you the following report recently conducted by the Phthalate Esters Panel.

**FINAL REPORT: 65-Week Repeated Oral Dose Toxicity Study
of DEHP in Juvenile Common Marmosets. March 17, 2003**

Please note, the report does not include confidential information. If you have any questions, please contact me.

Sincerely yours,

Contain NO CBI

Marian K. Stanley
Senior Director, CHEMSTAR
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Enclosure

267154



B000496

FINAL REPORT

Sixty-Five-Week Repeated Oral Dose Toxicity Study of
Di(2-ethylhexyl)phthalate (DEHP) in Juvenile Common Marmosets

(Study No.B000496)

March 17, 2003

Mitsubishi Chemical Safety Institute Ltd.

267154

STATEMENT

Sponsor: Japan Plasticizer Industry Association

Title: Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate (DEHP) in Juvenile Common Marmosets

Study Number: B000496

This study in this report was conducted in compliance with the following Good Laboratory Practice Standards.

- OECD GLP (1997)
- Japanese GLP on Industrial Chemicals (1984, 1988, 2000)

Study Director:

Yoshimasa Kurata
Yoshimasa Kurata, DVM.

Date: *March 17, 2003*

Toxicology Division, Kashima Laboratory
Mitsubishi Chemical Safety Institute Ltd.

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STUDY OUTLINE

1. **Title:** Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethyl-hexyl)phthalate (DEHP) in Juvenile Common Marmosets
(Study number:B000496)
2. **Purpose:** The purpose of this study is to characterize the potential toxic effects of DEHP on the development of the reproductive organs in juvenile common marmosets and to characterize plasma concentration, excretion, and tissue distribution of DEHP.
3. **GLP:** OECD GLP (1997)
Japanese GLP on Industrial Chemicals (1984, 1988, 2000)
4. **Sponsor:** Japan Plasticizer Industry Association
5-26, Motoakasaka 1-chome, Minato-ku, Tokyo, Japan
<Sponsor representative> Yoshio Oka
5. **Organization under Contract:**
Mitsubishi Chemical Safety Institute Ltd.
1-30, Shiba 2-chome, Minato-ku, Tokyo, Japan
6. **Testing Facility:** Kashima Laboratory
Mitsubishi Chemical Safety Institute Ltd.
14, Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, Japan
<Hormonal analysis>
Panapharm Laboratories, Co., Ltd. (Kumamoto, Japan)
<Zinc analysis>
Yokohama Laboratory, Mitsubishi Chemical Safety Institute Ltd.
(Kanagawa, Japan)
7. **Study Director:** Yoshimasa Kurata, DVM.
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(Clinical Examination)

Naoto Toyota

(Pathological Examination)

Yuki Tomonari

(Analysis of Liver and Testicular Contents)

Hiroshi Iwata

(Chemical Analysis of Dosing Solution)

Yoshie Suzuki

(Pharmacokinetic Study)*

Michinori Okada

*: Pharmacokinetic Study was conducted using another study number (Study No. B001107). This report includes the results of the pharmacokinetic study as SECTION-II.

9. Study Contributors:

SECTION-I < Repeated dose toxicity study >

(Assignment of Animals)

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(Animal maintenance)

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Mayumi Kitami, Masato Sudo, Fumie Miyazawa, Kayo Igarashi,
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(Clinical Observation)

Yoshimasa Kurata, Takeshi Kawasuso, and Fumie Miyazawa

(Body Weight Measurement)

Yoshimasa Kurata and Takeshi Kawasuso

(Blood Collection for Hormonal Analysis, Hematology, and Blood Chemistry)

Yoshimasa Kurata, Takeshi Kawasuso, Mayumi Kitami,
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Naoto Toyota, Ayako Mizushima, Junko Hoki, Katsuyo Takano,
Midori Ooe, and Natsumi Hanaka

(Hormonal Analysis)

Yukihiro Nakashima (Panapharm Laboratories, Co., Ltd.,
Kumamoto, Japan)

(Urine Collection)

Yoshimasa Kurata, Takeshi Kawasuso, and Kayo Igarashi

(Anesthetization)

Yoshimasa Kurata, Takeshi Kawasuso, Shinobu Matsumoto,
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Yukari Sato, Mayumi Iwai, Einosuke Tani, Shinobu Matsumoto,
Takeshi Kawasuso, Keiko Tada, Takuya Doi, Osamu Katsuta,
Yoshimasa Kurata, and Yuki Tomonari

(Organ Weight Measurement)

Takeshi Kawasuso, Shinobu Matsumoto, and Yoshimasa Kurata

(Tissue Preparation for Histopathological Examination)

Megumi Yamamoto, Miki Kozasa, Yuki Tomonari,
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(Histopathological Examination)

Yuki Tomonari

(Tissue Preparation for Electron-microscopic Examination)

Yuki Tomonari and Takayoshi Ito

(Electronmicroscopic Examination)

Yuki Tomonari

(Measurement of Enzyme Activities and Contents in the Testis and Liver)

Yoshie Yamamoto, Yoshihito Ishizuka, Yoshiharu Osawa,
Mikako Torii, Tomokazu Onuma, Takehiko Okuma, and
Tetsuya Kaneko

December 7, 2001

Termination of the experiment

March 14, 2002

End of the study

March 17, 2003

- 11. Retention:** The protocol, specimens, raw data, the test article, documents, and the final report will be retained in the archives of the Kashima Laboratory. They will be retained for the period of 10 years after submission of the final report and further retention will be discussed with the sponsor.
- 12. Ethics:** The protocol of this study was reviewed by the Committee for Ethics in Animal Studies in Kashima Laboratory in compliance with "Guidelines for Animal Studies (Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd.)".

STUDY DIRECTOR SIGNATURE

Sponsor: Japan Plasticizer Industry Association

Title: Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate (DEHP) in Juvenile Common Marmosets

Study Number: B000496

Study Director: Yoshimasa Kurata Date: March 17, 2003
Yoshimasa Kurata, DVM.
Toxicology Division, Kashima Laboratory
Mitsubishi Chemical Safety Institute Ltd.

SUMMARY

DEHP was administered by oral gavage at doses of 0, 100, 500, and 2500 mg/kg for 65 weeks to juvenile common marmosets (about 3 months old) of both sexes, and its toxicity was assessed. Additionally, pharmacokinetic parameters such as blood levels, urine and feces excretions, or tissue distributions were examined using a ring-labeled DEHP in juvenile (about 3 months old) and adult (about 18 months old, with or without DEHP-pretreatment) animals. An extensive and intensive investigation focused on testicular morphology, function, and toxicokinetics.

<Repeated dose toxicological study>

As the results, treatment-related change in the body weight was not evident. Treatment-related changes were not observed in general except for adaptive liver changes.

During the treatment period, all males experienced a surge in testosterone, and the testosterone levels in all treated groups were similar to that of the control group. For the testis, electron microscopic examination was additionally applied, however, this revealed no treatment-related abnormalities. Histochemical examination after 3 β -hydroxysteroid dehydrogenase (3 β -HSD) staining did not reveal any alteration in steroid synthesis. Consumption of peroxide scavenger like GSH, GST, or GSH-Px in the testis was not noticed, which suggests that the peroxysomal enzyme may not be operating in this organ. For functional examination, sperm count was conducted to show no treatment related effect in numerical changes.

The liver weight and its body weight ratio were not affected. P450 content tended to increase with dose-dependent manner in general, and that was considered to be adaptive change to the DEHP-exposure. However, with regard to specific CYP, CYP3A and 2E these are related to testosterone 6 β hydroxylation and lauric acid ω 1 hydroxylation, respectively, and CYP4A related to lauric acid ω hydroxylation, no marked increases were noticed. That showed induction of non-PP dependent oxidation and proved absorption of the test substance.

In the toxicological study, despite the high dose of 2500mg/kg/day and adoptive liver change proving absorption, no testicular change was morphologically or functionally noticed in the extensive examinations.

<Pharmacokinetic study>

Evidence of absorption was also provided from the pharmacokinetic study. When

aromatic ring-labelled DEHP was given to the common marmoset, high concentration in bile was observed and considered one of predominant pathways in excretion. Distribution study showed that concentration in the bile was 36-47 times as much as that in the blood. Entero-hepatic circulation was considered to be involved. Fecal excretion that exceeds the urinary route did not necessary mean the evidence of poor absorption in marmosets. Meanwhile, the testis showed c.a. 1/10 and the liver showed similar R.I. level as much as the blood concentration, suggesting limited accumulation of DEHP metabolite(s) in testes.

In conclusion, when DEHP was administered orally to juvenile marmoset at dosage levels of 100, 500, and 2500 mg/kg/day for 65 weeks until maturation, the testicular effect that is well known in rodents were not observed despite extensive examination. The apparent mechanism of the species difference may be related to the evidence of limited accumulation of the metabolite in testes.

SECTION-I <Repeated Dose Toxicity Study>

MATERIALS AND METHODS

1. *Test article*

- 1.1 Name:** Di(2-ethylhexyl) phthalate (DEHP, >98%) [CAS:117-81-7]: for SECTION-I and SECTION-II
- 1.2 Lot number:** FGG01 and FGJ01
- 1.3 Purity:** 99.6% for the both lots, FGG01 and FGJ01
- 1.4 Supplier:** Tokyo Chemical Co., Ltd. (Tokyo, Japan)
- 1.5 Storage condition:**
The test article was stored in a well-closed bottle protected from light at room temperature (Actual temperature: 11.0° to 28.0°C).
- 1.6 Confirmation of the stability:**
The test article was confirmed to be stable during the experimental period by the analytical report obtained from the supplier, Tokyo Chemical Co. Ltd. (Tokyo, Japan).

2. *Vehicle**

- 2.1 Reagent:** Corn oil (Lot No. 9J2240, OH2195, 1A2136, 1B2144, and 1I2140, Junsei Chemical Co., Ltd.)
- 2.2 Storage condition:**
The vehicle was stored under the same conditions with the test substance.

*: The vehicle was used as a dosing solution of the control group.

3. *Experimental Animals*

- 3.1 Species:** Common marmoset (*Callithrix jacchus*)

3.2 Reasons for selection of the species:

The common marmoset (*Callithrix jacchus*) is widely used for toxicity studies related to reproductive performance because of the resemblance to humans in this aspect, and there are plenty of historical data, and a large number of animals are available.

3.3 Supplier: CLEA JAPAN Inc. (Tokyo, Japan)

3.4 Microbial level: Conventional

3.5 Number of animals:

Age matched animals were supplied batch by batch and treatment was started sequentially. Because the commencement of handling started in an early life stage as possible, some animals randomly succumbed and the occurrences of moribund wasting were not considered to be dose dependent. When it occurred, the animal was replaced with a new animal to fulfil the object of the study.

Total number of animals purchased for the study was 74 male and 56 females.

3.6 Quarantine plus acclimatization period:

Because one of the purpose of the study was to start administration as early in the life study as technically possible, animals were carried in immediately after weaning and minimum quarantine plus acclimatization period of 2 weeks.

3.7 Age at the commencement of treatment:

About 100 days old (range: from 90 to 110 days).

Although, two female animals (#50203 and #60201) eventually exceeded the range and administration started at 115 or 112 day old, the animals were considered valid.

3.8 Body weight on the day of initiation of dosing:

Males: 95 – 180 g

Females: 116 – 188 g

3.9 Assignment:

The animals were allocated to 4 groups each comprising of 9 males and 6 females on the basis of the results of pre-examination (clinical observation and body weight measurement). Animals unassigned to groups were excluded from the study. Succumbed animals were supplemented to achieve the required number of animals.

3.10 Identification of animals:

The animals were identified by the individual number given by the supplier on a collar tag. A code table was prepared for checking the consistency between the animal numbers assigned for this study and the individual numbers. In addition, each cage was identified with a label indicating the individual number, sex, quarantine number before assignment, and after assignment, study number, test article name, dose level, animal number, individual number, and sex.

4. *Animal husbandry*

4.1 Animal room number:

Room No.3126

4.2 Environmental conditions

4.2.1 Temperature: Actual value: 22.4° – 28.9°C
(Target value: 26.0°C, permissible range: 23.0° – 29.0°C)

4.2.2 Relative humidity:
Actual value: 32.5% – 100.0%
(Target value: 55.0%, permissible range: 35.0% – 75.0%)

4.2.3 Ventilation: 6 – 25 time/hours (all fresh filtered air)

4.2.4 Light cycle: 12 hours (light: 08:00 to 20:00)

4.3 Animal accommodations

4.3.1 Cages: Stainless-steel cages (300W × 600D × 650H mm, CLEA JAPAN Inc., Tokyo, Japan).
The cages were washed with tap water once a day.

4.3.2 Feeders: Polypropylene feeders (CLEA JAPAN Inc., Tokyo, Japan).
The feeders were replaced with ones that were sterilized with steam once a day.

4.3.3 Racks: Two-tiered stainless steel racks (CLEA JAPAN Inc., Tokyo, Japan).
The racks were washed with tap water once a day.

4.4 Food stuff

4.4.1 Type: CMS-1 and CMS-1M (CLEA JAPAN Inc., Tokyo, Japan), a solid chow for new-world primate

4.4.2 Additives: Ascorbic acid, powdered milk and water

4.4.3 Provision of diet:

An 80 g/head/day of the mixture of main diet and additives (200g of water and 1g of ascorbic acid for 1000g of pellet diet) prepared everyday was given every morning and replaced the next morning. Powdered milk (1 to 2g) was given to the animal that lacks appetite for the main diet.

4.4.4 Analysis of contaminant:

The dietary concentrations of contaminants such as pesticide residues were confirmed to be below the permissible limit specified at our laboratory. Data of every lot concerned with the nutrients and biological examination was obtained from a supplier.

4.5 Drinking water

4.5.1 Source: Tap water filtered using a 5- μ m filter and irradiated with ultraviolet rays was used.

4.5.2 Delivery: All animals were provided access to water *ad libitum* through a nozzle of the water delivery system (Tokiwa Kagaku, Tokyo, Japan).

4.5.3 Analysis of contaminant:

The drinking water was analyzed periodically, and the analytical values were confirmed to meet the specifications defined by the Waterworks Law of Japan.

4.6 Accommodation: One animal per cage

5. Administration

5.1 Route and method:

Oral administration by gavage with a gastric tube (nasal feeding catheter, Atom Medical Corporation, Tokyo, Japan) and glass syringe.

5.2 Reason for the selection of the route:

Oral intake is thought to be the main exposure-route in humans.

5.3 Reason for the selection of the method:

The method is generally used for marmosets when a solution is administered orally, and they can be treated accurately.

5.4 Dosing frequency and period:

The dosing suspension was administered once a day, 7 days per week for 65 weeks. Administration was discontinued for a while, when it is diagnosed that the animal could not tolerate the administration operation, by the veterinary observation. The dosing frequency for each animal, however, was more than 93.4% of predetermined times of administration.

5.5 Dosages and reason for the selection:

Three dose levels (100, 500, and 2500 mg/kg) except the control (0 mg/kg) were set by a common ratio of 5. The high doses were the maximum tolerated level and the maximum technically-possible level according to a preliminary study (Study No. B000495). The control group was given the vehicle alone.

5.6 Dose volume: Dose volume was set at 5 mL/kg weight, and that of individual animal was calculated based on the most recently body measured weight.

5.7 Dose preparation**5.7.1 Preparation method and frequency:**

The test substance was mixed with the vehicle, corn oil (Junsei Chemical Co., Ltd., Tokyo, Japan), containing 20, 100, and 500 mg/mL. The solutions were prepared on a weekly basis, and were stored in a refrigerator (target range: 2° to 15°C, actual range: 1.5° to 16.0°C) until dosing. They were used within 10 days.

5.7.2 Confirmation of the stability:

The formulation was confirmed to be stable in the former study (study no.: 4L209, Mitsubishi Chemical Safety Institute Ltd.) at 20 and 500 mg/mL for 10 days when stored in a refrigerator.

5.7.3 Confirmation of the concentration:

At the first preparation, the dose solutions were analyzed to confirm

each concentration. As a result, concentrations of the test substance in the vehicle were 102.0%, 102.0%, and 102.0% to the indicated concentrations, 20, 100, and 500 mg/mL, respectively.

6. Group Constitution

Test substance	Dose level (mg/kg)	Number of animal (Animal no.)		
		Group 1		Group 2
		Male	Female	Male
Vehicle	-	8 <5> ^{*1} (10101 ^{*2} , 10102, 10103, 10104, 10105, 10106 ^{*2} , 10110 ^{*3})	6 <6> ^{*1} (50101, 50102, 50103, 50104, 50105, 50106)	3 <3> ^{*1} (10107, 10108, 10109 ^{*2} , 10111 ^{*4})
DEHP	100	9 <6> ^{*1} (10201, 10202 ^{*2} , 10203 ^{*2} , 10204, 10205, 10206, 10210, 10211, 10213 ^{*5})	6 <6> ^{*1} (50201, 50202, 50203, 50204, 50205, 50206)	4 <3> ^{*1} (10207, 10208, 10209 ^{*2} , 10212)
DEHP	500	8 <7> ^{*1} (10301, 10302, 10303, 10304, 10305 ^{*2} , 10306, 10310 ^{*3} , 10311 ^{*3})	6 <6> ^{*1} (50301, 50302, 50303, 50304, 50305, 50306)	3 <3> ^{*1} (10307, 10308, 10309)
DEHP	2500	7 <6> ^{*1} (10401, 10402, 10403, 10404, 10405, 10406 ^{*2} , 10410 ^{*3})	6 <5> ^{*1} (50401, 50402 ^{*2} , 50403, 50404, 50405, 50406)	3 <3> ^{*1} (10407, 10408, 10409)

Group 1: Animals were necropsied routinely.

Group 2: The testis of the animals was perfused with glutaraldehyde solution before necropsy.

*1: The number of animals completed whole treatment period.

*2: These animals were omitted during the treatment period because of wasting symptom.

*3: These animals were supplemented to fill the omitted animals.

*4: This animal was moved from group 1. Basis for selection was that the animal was assigned to highest experiment number.

*5: This animal was a female and omitted during the treatment period.

Several animals (shown as *2) have succumbed during treatment period to wasting symptom such as marked weight reduction, loose feces, and diarrhea. These wasting animals appeared sporadically in the early stage of administration in non-dose-dependent way. Therefore, the occurrence was considered to be incidental and not to be treatment-related, and such wasting animals were omitted from the toxicity assessment and replaced with the animals shown as “*3”.

7. Observations and Examinations

7.1 Clinical examination and body weight measurement

All animals in Group 1 and 2, and Group 5 in the pharmacokinetic study were observed once daily for sign of any systemic reactions by assessing clinical symptoms and behavior, and were weighed at least once per week during the treatment period.

7.2 Hematological examination

At the scheduled sacrifice, blood samples were obtained from the femoral vein of all animals in Group 1 and 2. Using a portion of the blood samples, the following items were routinely determined by an auto analyzer. EDTA (dipotassium-salt) was used as an anticoagulant.

7.2.1 Erythrocyte counts:

Isovolumetrically sphered two optical cytometric laser FCM

7.2.2 Hemoglobin concentration:

Modified cyanmethemoglobin method

7.2.3 Hematocrit: Isovolumetrically sphered two optical cytometric laser FCM

7.2.4 Mean corpuscular volume (MCV):

Calculated from the erythrocyte count and hematocrit

7.2.5 Mean corpuscular hemoglobin (MCH):

Calculated from erythrocyte count and hemoglobin conc.

7.2.6 Mean corpuscular hemoglobin concentration (MCHC):

Calculated from hemoglobin concentration and hematocrit

7.2.7 Reticulocyte ratio:

RNA-stained laser FCM

7.2.8 Platelet count: Isovolumetrically sphered two optical cytometric laser FCM

7.2.9 Leukocyte counts:

Peroxidase cytometric FCM and Baso/lobularity laser FCM

7.2.10 Leukocyte count-differential:

Counted after Wright's stain

[Measuring instruments]

ADVIA120, Bayer Medical Ltd.: 7.2.1-7.2.9

MICROX HEG-50VF and HEG-50, Omron Corporation (Kyoto, Japan): 7.2.10

7.3 Blood chemical examination

Another portion of the blood sample obtained at the scheduled sacrifice was collected in heparinized (lithium-salt) tubes. Then the plasma was separated by centrifugation and routinely examined for the following items.

7.3.1 ASAT: UV-rate method (JSCC modified method)

- 7.3.2 ALAT: UV-rate method (JSCC modified method)
- 7.3.3 γ -glutamyl transpeptidase (γ -GT):
 γ -glutamyl-p-nitroanilide substrate method (SSCC modified method)
- 7.3.4 Lactate dehydrogenase (LDH):
UV-rate method (modified JSCC method)
- 7.3.5 Alkaline phosphatase (ALP):
P-nitrophenylphosphate substrate method (JSCC modified method)
- 7.3.6 Total bilirubin: Enzymatic method (BOD method)
- 7.3.7 Urea nitrogen: Enzymatic-UV method (urease-LEDH method)
- 7.3.8 Creatinine: Enzymatic method (creatine kinase method)
- 7.3.9 Glucose: Enzymatic-UV method (GlcK-G6PDH method)
- 7.3.10 Total cholesterol:
Enzymatic method (CO-HDAOS method)
- 7.3.11 Free cholesterol: Enzymatic method (CO-POD method)
- 7.3.12 Triglyceride: Enzymatic method (GPO-HDAOS method)
- 7.3.13 Phospholipid: Enzymatic method (COD-DAOS method)
- 7.3.14 Total protein: Biuret method
- 7.3.15 Albumin: BCG method
- 7.3.16 A/G ratio: Calculated from total protein and albumin
- 7.3.17 Calcium (Ca): OCPC method
- 7.3.18 Inorganic phosphate (IP):
UV method (PNP-XOD-POD method)
- 7.3.19 Sodium (Na): Ion selective electrodes method
- 7.3.20 Potassium (K): Ion selective electrodes method
- 7.3.21 Chloride (Cl): Ion selective electrodes method

[Measuring instruments]

Automatic analyzer (TBA-200FR, Toshiba Co., Ltd. Tokyo, Japan): 7.3.1-7.3.21

7.4 Hormonal analysis:

Prior to and 13, 26, 39, 52, and 65 weeks after commencement of the treatment, blood samples were obtained from the femoral vein of all animals in Group 1 and collected in heparinized (lithium-salt) tubes. Then the plasma was separated by centrifugation and examined for the following items. The plasma samples were stored at -80°C (acceptable range: below -70°C) until analysis.

Analysis of the following items was contracted to Panapharm laboratories Inc. (Kumamoto, Japan). Peptide hormones such as Follicle-stimulating hormone and luteinizing hormone were undetectable due to lack of cross-reactivity.

7.4.1 Testosterone: Radioimmunoassay

7.4.2 Estradiol (E2): Radioimmunoassay

7.4.3 Triiodothyronine (T3) and thyroxine (T4):
Radioimmunoassay

7.5 Necropsy:

On the day after final dosing, all animals in Group 1 and 2 were sacrificed by exsanguination from the abdominal aorta and vein under pentobarbital anesthesia, and subjected to necropsy. Animals in Group 2 were perfused with glutaraldehyde solution systematically just before exsanguination.

7.6 Tissue fixation or storage method:

Groups	Sex (n)	Organs	Fixation	Examination	Ref.
Group 1	Male (6)	Testis (L) and Epididymis (L)	Bouin's sol.	Light microscopy	7.7
		Testis(R)	Freezing ^a	Enzyme and component analysis Sperm counting	7.10
		Epididymis (R)	Freezing ^a	Sperm counting	7.10
	Female (6)	Liver lobe	Freezing ^a	Enzyme and component analysis	7.11
		Others	Formalin sol.	Light microscopy	7.7
		Ovary (L)	Freezing ^a	Histo-chemistry	7.8
		Liver lobe	Freezing ^a	Enzyme and component analysis	7.11
		Others	Formalin sol.	Light microscopy	7.7
		Group 2 (Male) (3)	Testis (L) and Epididymis (L)	Freezing ^a	Histo-chemistry
Testis (R) and	Perfusion with glutaraldehyde sol.		Electron microscopy	7.9	
Others	Formalin sol.		Light microscopy	7.7	

- Group 1: Animals were routinely necropsied.

- Group 2: The animals were systemically perfused with glutaraldehyde at necropsy.

- ^a: The tissue was quickly frozen in liquid nitrogen and stored at -80°C (acceptable range: below -70°C) until analysis.

- L: Left, R: Right

7.7 Light microscopic examination:

Light microscopic examination was performed on the sections of the following organs from all animals in Groups 1 and 2 (except testes from animals in Group 2). The sections were routinely stained with hematoxylin and eosin. All organs were weighed and the ratio of organ weight to body weight were then calculated.

- | | | | |
|--------------------------|--------------------------------|------------|--------------|
| – Pituitary | – Thyroid | – Pancreas | – Liver |
| – Adrenal | – Kidney Spleen | – Testis | – Epididymis |
| – Prostrate ^a | – Seminal vesicle ^a | – Ovary | – Uterus |

^a: without fluid

7.8 Histochemical examination of the testes and ovaries:

A 3 β -hydroxysteroid dehydrogenase (3 β -HSD) activity in the testes or ovaries from all animals in Groups 1 (females) and 2 (males) was examined histo-chemically, according to Rune G et al. (1991) and Rune G M. and Heger W. (1987).

Regarding the testis, the activity of 3 β -HSD in Leydig cell was classified in 4 grades on 100 fields of view, and then the mean positive area in each grade was calculated. Regarding the ovary, the ratio of positive area against whole area was measured using an image analyzer (IPAP, Sumika-Technos, Osaka, Japan).

7.9 Electron microscopic examination of the testis:

As shown above, the testis from all males in Group 2 were perfused with glutaraldehyde solution through the left ventricle and aorta under pentobarbital anesthesia just before necropsy, and then fixed in osmium tetroxide solution. Electron microscopic examination was performed on the thin sections double-stained with uranyl acetate and lead citrate using a electron microscope (JEM-100CX II STEM microscope, Nihon Denshi Co., Ltd.). Special attention was paid, among others, on Sertoli cell organelles like rough endoplasmic reticulum (rER) near junction, ribosome or Leydig cell for any change in organelle peroxisome.

7.10 Testicular component and enzyme activities

A portion of the testis from all males in Group 1 was obtained at first and used for zinc analysis (7.10.7). Using the residue of the tissue, about 25w/v% homogenate was prepared with 1.15w/v% KCl using a high-speed homogenizer (Polytron[®]: Kinematica GmbH, Switzerland) and a portion of this homogenate was used for the sperm count (7.10.1). The residue of the homogenate was centrifuged (9000 \times g, 20 min., at 4°C) to obtain a supernatant (S-9) and this S-9 fraction was used for measurements of the following enzyme activities or contents (7.10.2 to 7.10.6). A protein content of the S-9 fraction was measured by a Lowry method using a spectrophotometer (DU-7400: Beckman, Germany).

7.10.1 Sperm count:

The testis was homogenized with 1.15% KCl solution, and the sperm head was measured using a hemocytometer. The result was expressed in number of head/g testes.

7.10.2 Sorbitol dehydrogenase (SDH)

The activity was measured by an UV-rate method using an automatic analyzer (TBA-200FR, Toshiba Corporation, Japan). The result was expressed in $\mu\text{mol/g}$ wet tissue.

7.10.3 γ -glutamyl transpeptidase (γ -GT)

The activity was measured by a γ -glutamyl-p-nitroanilide substrate method (SSCC modified method) using an automatic analyzer (TBA-200FR, Toshiba Corporation, Japan). The result was expressed in $\mu\text{mol/g}$ wet tissue the testis and $\mu\text{mol/mg}$ protein of the S-9.

7.10.4 Glutathione content

Total content (GSH + GSSG) was measured by the DTNB-GR method using a spectrophotometer (DU-7400: Beckman, Germany). The result was expressed in $\mu\text{mol/g}$ wet tissue.

7.10.5 Glutathione S-transferase (GST)

The GST activity was measured by the CDNB (1-chloro-2,4-dinitrobenzene) substrate method using a spectrophotometer (DU-7400: Beckman, Germany). The result was expressed in $\mu\text{mol/g}$ wet tissue of the testis/min. and $\mu\text{mol/mg}$ protein of the S-9/min.

7.10.6 Glutathione peroxidase (GSH-Px)

The GSH-Px activity was measured by a commercially available kit (tart-butyl hydroperoxide substrate method, BIOXYTECH[®] GPX-340[™]: Oxis International, Inc., USA) using a spectrophotometer (U-3310: Hitachi, Ltd., Japan). The result was expressed in $\mu\text{mol/g}$ wet tissue of the testis/min. and $\mu\text{mol/mg}$ protein of the S-9/min.

7.10.7 Zinc (Zn) content

Sample tissue of the testis was decomposed by heating with nitric acid and hydrogen peroxide solution. Then, zinc content was determined by a flame-less atomic absorption spectrophotometer (SpectraAA 880Z, Varian, U.S.A.).

Analysis of this item was contracted to Yokohama Laboratory of Mitsubishi Chemical Safety Institute Ltd. (Kanagawa, Japan).

7.11 Hepatic enzyme analysis:

A portion of the liver from all animals in Group 1 was used for measurements of the following enzyme activities or contents. About 25 w/v% homogenate was prepared with

1.15w/v% KCl using a glass-Teflon homogenizer and a portion of this whole-homogenate was used for measurements of peroxisomal enzyme activities (7.11.1 to 7.11.3). The residue of the homogenate was centrifuged (9000 \times g, 20 min., 4°C) to obtain a supernatant (S-9) and a portion this S-9 fraction was used for measurements of glutathione content and glutathione peroxidase activity (7.11.8 to 7.11.9). The residue of the S-9 fraction was centrifuged (105000 \times g, 60 min., 4°C) to obtain cytosol and microsome fractions. This cytosol fraction was used for a measurement of glutathione S-transferase (7.11.7) and the microsome fraction was re-suspended in 0.1 mmol/L EDTA-2Na, 10 mmol/L potassium-phosphate buffer (pH 7.4), and used for measurements of P-450 content, testosterone hydroxylation, and lauric acid hydroxylation (7.11.4 to 7.11.6). Protein contents of the whole-homogenate, S-9, cytosol, and microsome fractions were measured by the Lowry method using a spectrophotometer (DU-7400: Beckman, Germany and U-3210 and U-3310: Hitachi, Ltd., Japan).

7.11.1 Cyanide-insensitive palmitoyl co-A β -oxidation (FAOS)

FAOS activity was measured according to the method of Lazarow and De Duve (1976) using palmitoyl CoA as the substrate and a spectrophotometer (U-3210: Hitachi, Ltd., Japan). The result was expressed in μ mol/g wet tissue of the testis/min. and μ mol/mg protein of the whole-homogenate/min.

7.11.2 Carnitine acetyltransferase (CAT)

CAT activity was measured according to the method of Markwell *et al.* (1973) using acetyl CoA as the substrate and a spectrophotometer (U-3310: Hitachi, Ltd., Japan). The result was expressed in μ mol/g wet tissue of the testis/min. and μ mol/mg protein of the whole-homogenate/min.

7.11.3 Carnitine palmitoyltransferase (CPT)

CPT activity was measured according to the method of Markwell *et al.* (1973) using a palmitoyl CoA as the substrate and a spectrophotometer (U-3310: Hitachi, Ltd., Japan). The result was expressed in μ mol/g wet tissue of the testis/min and μ mol/mg protein of the whole-homogenate/min.

7.11.4 Cytochrome P-450 content

P-450 content was measured according to the method of Omura and Sato (1964) using a spectrophotometer (U-3310: Hitachi, Ltd., Japan). The result was expressed in nmol/g wet tissue and pmol/mg microsomal protein.

7.11.5 Testosterone 6 β -hydroxylation

The testosterone 6 β -hydroxylation activity was measured by the testosterone substrate method using an HPLC system (D-7000 Series: Hitachi, Ltd., Japan). The result was

expressed in nmol/g wet tissue/min and nmol/mg microsomal protein/min.

7.11.6 Lauric acid ω - and ω 1-hydroxylation

The activity was measured by the 14 C-lauric acid substrate method using a HPLC system (D-7000 Series: Hitachi, Ltd., Japan). The result was expressed in nmol/g wet tissue/min and nmol/mg microsomal protein/min.

7.11.7 Glutathione S-transferase (GST)

The activity was measured by the CDNB (1-chloro-2,4-dinitrobenzene) substrate method using a spectrophotometer (DU-7400: Beckman, Germany). The result was expressed in μ mol/g wet tissue/min and μ mol/mg cytosolic protein/min.

7.11.8 Glutathione

Total content (GSH + GSSG) was measured by the DTNB-GR method using a spectrophotometer (DU-7400: Beckman, Germany). The result was expressed in μ mol GSH eq/g wet tissue.

7.11.9 Glutathione peroxidase (GSH-Px)

The activity was measured by a commercially available kit (tart-butyl hydroperoxide substrate method, BIOXYTECH[®] GPX-340TM: Oxis International, Inc., USA) using a spectrophotometer (U-3310: Hitachi, Ltd., Japan). The result was expressed in μ mol/g wet tissue/min and nmol/mg S-9 protein/min.

7.12 Statistical analysis:

The metric data were first analyzed by Bartlett's test. When the group variances are determined to be homogeneous, all groups were compared by analysis of variance (ANOVA). Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) were used when inter-group differences were found to be significant. When the result of Bartlett's test indicated heterogeneous group variances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).

RESULTS

1. *Fate of animals*

Deaths or moribund sacrifices occurred in all groups including the control as showed in the following table. All of deaths or moribund sacrifices were considered not to be specific to the test-substance treatment. Therefore, additional animals were subjected to the treatment instead of dead or sacrificed ones except for one male (#10109) receiving the vehicle.

Test substance	Dose level (mg/kg)	Group	Animal No. (Days of treatment)	
			Male	Female
Vehicle	-	1	10101 (Day 106), 10106 (Day 63)	-
		2	10109 (Day 363)	-
DEHP	100	1	10202 (Day 106), 10203 (Day 119)	-
		2	10209 (Day 85)	-
DEHP	500	1	10305 (Day 116)	-
		2	-	-
DEHP	2500	1	10406 (Day 119)	-
		2	-	50402 (Day 129)

2. *Clinical signs and body weigh (Table 1, Appendix 1 and 2)*

There were no treatment-related abnormalities in any group.

Loose stool, reddish stool, or diarrhea was occasionally observed especially in the early stage of the treatment period. However, there was no difference in its incidence among all groups.

Dose related change in the body weight was not evident, except the wasting animals that showed marked decrease in body weight.

3. *Hematology (Table 2, Appendix 3)*

There were no treatment-related abnormalities in any group.

4. *Blood chemistry (Table 3, Appendix 4)*

There were no treatment-related abnormalities in any group.

5. *Hormonal analysis (Table 4, Appendix 5)*

There were no treatment-related abnormalities in any group.

During the treatment period, all males experienced testosterone surge, and the testosterone levels in all treated groups were similar to that of the control group.

6. Organ weight (Table 5 and 6, Appendix 6 and 7)

There were no treatment-related abnormalities in any group.

A statistically significant high value was observed in female reproductive organs such as ovary and uterus in the 500- and 2500-mg/kg groups. However, no histological abnormality was observed.

One animal in each DEHP-treatment group of Group 1 showed low weights in the testis, epididymis, seminal vesicle, and prostate. These animals usually showed low body weight and such animal as showing low body weight and small size in above organs also existed in the control animals of Group 2.

7. Sperm count (Table 7, Appendix 8)

There were no treatment-related abnormalities in any group.

In the growing testis, low value of sperm count was observed as expected. The occurrence of the growing animal is not dose dependent. To examine the trend of administration effect on sperm count, inclusion of exceptional incidence was avoided. Namely, data from animals that were diagnosed as "growing" (#10204, #10302 and #10402) were omitted from Table 7.

8. Testicular enzyme activities and component (Table 8, Appendix 9)

There were no treatment-related abnormalities in any group.

9. Hepatic enzyme activities and component (Table 9, Appendix 10)

P450 content tended to increase with dose-dependent manner in general with statistical significance in females at 500 and 2500 mg/kg. With regard to specific CYPs, CYP3A and 2E these were related to testosterone 6 β hydration and lauric acid ω 1 hydration and CYP4A that is related to lauric acid ω hydration, no marked or specific increase was noticed.

Total GSH content also tended to decrease with dose-dependent manner in general. However, these changes were considered to be minimal.

On the other hand, there were no changes in the peroxisomal enzyme activities such as CAT, CPT, and FAOS.

10. Necropsy finding (Table 10, Appendix 11)

There were no treatment-related abnormalities of the testis in any group.

One or 2 males (#10108, #10204, #10208, #10302, #10307, #10401, and #10402) in each group showed small size of genital organs such as testis, epididymis, seminal vesicle, prostate. However, this change was considered to not be a primary effect of the test substance but due to low body weight.

Large size of the ovary was observed in 2 females each receiving 500 and 2500 mg/kg of DEHP, no abnormality, however, was observed histopathologically.

Dark-brownish change of the liver was observed in 1 male receiving 2500 mg/kg of DEHP and may be treatment related. However, no change was evidenced in the histopathological examination.

11. Histological findings (Table 11, Appendix 12)

There were no treatment-related abnormalities in the organs examined from animals in any group.

Testis, epididymis, seminal vesicle, and prostate with small size due to low body weight did not mature satisfactorily (on growing). However, degenerative change ascribed to the DEHP-treatment was not observed in the above growing testis.

In the ovary with large size in 2 females each receiving 500 and 2500 mg/kg of DEHP, a development of the corpus luteum was noticed, however such corpus luteum is usually observed in more mature females.

12. Histological findings (3 β -HSD) (Table 12, Appendix 13)

There were no treatment-related abnormalities in the testis or ovary from animals in any group.

Lydig cells in the testis from the animals treated with DEHP were strongly positive to 3 β -HSD staining as well as that in control animals. However, in the growing testis, 3 β -HSD activity was undetectable as expected.

The corpus luteum in the ovary was well stained by 3 β -HSD immunostaining, so the large variation in the positive area in the ovary, which increased in a dose dependent manner, depends on the development of the corpus luteum.

13. Electron microscopic findings (Table 13)

There were no treatment-related abnormalities in the Lydig cells, Sertoli cell, or

spermatic cells in the testis from animals in any group.

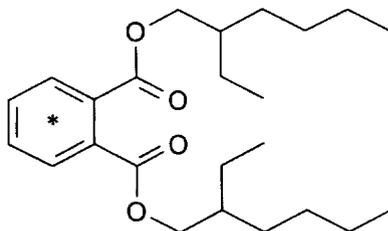
In this examination, the observation was focused especially on Sertoli cell. However, vacuolative change of rER (rough endoplasmic reticulum) near the tight junction or destruction of the desmosome that were observed in rodents were not observed in marmosets.

SECTION-II <Pharmacokinetic study>**MATERIALS AND METHODS****1. Test substances**

1.1 Name: ^{14}C -DEHP

1.2 Chemical name: Bis(2-ethylhexyl) [ring- ^{14}C]phthalate

1.3 Structural formula:



*: ^{14}C -labeled position

1.4 The radiochemical purity:

The radiochemical purity was determined before the first use and confirmed.

1.5 Storage condition:

Frozen (-20°C) and shielded from light

1.6 Stability:

The radiochemical purity was determined after the final use and confirmed.

1.7 Lot number 059F9253

1.7.1 Origin: Supplied by Exxon-Mobil Biomedical Sciences, Inc. for this study.

1.7.2 Specific activity:
25.9 mCi/mmol (2.45 MBq/mg)

1.7.3 Property: Colorless liquid

1.7.4 Special mention: Because of a decline of the radiochemical purity, it was entrusted to Daiichi Pure Chemicals Co., Ltd. and was refined and used. Lot number after the refinement was given as CP2622.

1.8. Lot number CFQ11987

- 1.8.1 Origin: Synthesized by Amersham Pharmacia Biotech as a test substance used in the previous study "Rat placenta, fetal transitory study"(B000272). The lot kept in a freezer (-20°C) after usage in B000272 was used in the study.
- 1.8.2 Specific activity:
144.3 MBq/mmol (366 kBq/mg)
- 1.8.3 Property: Colorless liquid

1.9 Non-labeled compound

- 1.9.1 Chemical name :
Di(2-ethylhexyl) phthalate [CAS: 117-81-7]
- 1.9.2 Abbreviated name:
DEHP
- 1.9.3 Supplier: Tokyo Chemical Co., Ltd.
- 1.9.4 Lot number: FGJ01.
The same lot as the substance used in the study "Sixty Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl) phthalate (DEHP) in Juvenile Common Marmosets (study No. B000496)" was used in the study.
- 1.9.5 Property: Colorless liquid
- 1.9.6 Purity: 99.6%
- 1.9.7 Storage condition:
At room temperature
- 1.9.8 Stability: The stability (purity) of the substance was confirmed by Tokyo Chemical Co. Ltd. in the lot used in the study (study No. B000496) before and after dosing.

2. Experimental Animals

- 2.1 Species: Common marmoset (*Callithrix jacchus*)
- 2.2 Supplier: CLEA JAPAN Inc. (Tokyo, Japan)

2.3 Microbial level: Conventional**2.4 Juvenile animals (Group 3)**

2.4.1 Number of animals:

Six males and six females

2.4.2 Age at the commencement of treatment:

About 3 months old

2.4.3 Body weight on the day of initiation of dosing:

Males: 110 – 139 g

Females: 128 – 172 g

2.5 Adult animals (Group 4)

2.5.1 Number of animals:

Seven males and seven females

2.5.2 Age at the commencement of treatment:

18 months old

2.5.3 Body weight on the day of dosing:

Males: 269 – 414 g

Females: 249 – 373 g

2.6 Adult animals with pretreatment of DEHP (Group 5)

2.6.1 Number of animals:

Six males and six females, which had been pretreated with DEHP for 65 weeks as same as SECTION-I.

2.6.2 Age at the commencement of treatment:

18 months old

2.6.3 Body weight on the day of initiation of dosing:

Males: 250 – 322 g

Females: 201 – 351 g

2.7 Assignment: Regarding Groups 3 and 4, the animals were assigned to give approximately equal group mean body weight and the age on the last day of the acclimation period, respectively. Littermates were distributed equally among the groups. Regarding Groups 5, the grouping was performed in the toxicological study (SECTION-I),

and the description of quarantine and acclimation period and the method of an animal care during unlabeled DEHP administration period were shown in SECTION-I. Animals unassigned to groups were excluded from the study.

2.8 Identification of animals:

The animals were identified by the individual number given by the supplier on a collar tag. In addition, each cage was identified with a label indicating the individual number, sex, quarantine number before assignment, and after assignment, study number, test article name, dose level, animal number, individual number, sex, group name and cage number.

3. Animal husbandry

3.1 Animal room number:

Room No. 5118 and 5119

3.2 Environmental conditions

3.2.1 Temperature: Actual value: 22.3° – 30.8°C
(Target value: 26 ± 2°C, permissible range: 23.0° – 29.0°C)

3.2.2 Relative humidity:
Actual value: 26.0% – 60.2%
(Target value: 55 ± 15%, permissible range: 35.0% – 75.0%)

3.2.3 Ventilation: 12 time/hours (all fresh filtered air)

3.2.4 Light cycle: 12 hours (from 8:00 to 20:00) (except for lighting about 30 minutes for animal care and examination after 20:00)

3.3 Animal accommodations:

3.3.1 Cages: Metabolic cages made of stainless steel (250W×250D×420H mm, CLEA JAPAN Inc., Tokyo, Japan) was used and washed daily with tap water.

3.3.2 Feeders: Feeders made of stainless steel (CLEA JAPAN Inc., Tokyo, Japan) was used and washed daily with tap water.

3.3.3 Water-feeding bottle:
Water-feeding bottle made from polypropylene (500 mL,

NASTUME SEISAKUSYO Inc.) was used and washed daily with tap water.

3.4 Food Stuff:

3.4.1 Type: CMS-1 and CMS-1M (CLEA JAPAN Inc., Tokyo, Japan), a solid chow for new-world primate

3.4.2 Additives: Ascorbic acid and water

3.4.3 Provision of diet:

50 g/head/day of the mixture of main diet and additives (200 g of water and 1 g of ascorbic acid for 1000 g of pellet diet) prepared everyday was given every morning.

3.4.4 Analysis of contaminant:

The dietary concentrations of contaminants such as pesticide residues were confirmed to be below the permissible limit specified at our laboratory. Data of every lot concerned with nutrients and biological examination was obtained from a supplier.

3.5 Drinking water

3.5.1 Source: Tap water filtered using a 5 μ m filter and irradiated with ultraviolet ray was used.

3.5.2 Delivery: Provided *ad libitum* by a water-feeding bottle and exchanged daily with a new one.

3.5.3 Analysis of contaminant:

The drinking water was analyzed periodically, and the analytical values were confirmed to meet the specifications defined by the Water Works Law of Japan.

3.6 Accommodation: One animal per cage

4. Administration

4.1 Route: Oral administration

4.2 Incidence: Single

4.3 Method: The test substance was administrated by gavage into the stomach

using a nutrient catheter (4Fr, Atom Medical Corporation, Tokyo, Japan) connected to a glass injection tube (2 mL, Natsume Seisakusho Co., Ltd., Tokyo, Japan).

4.4 Dose levels: 100 and 2500 mg/kg

4.5 Radioactive dose

4.5.1 Juvenile animals (3 months old, Group 3):

About 10 MBq/kg

4.5.2 Adult animals (18 months old, Group 4 and Group 5):

About 5 MBq/kg

4.6 Dose volume: Dose volume was set at 5 mL/kg, and that of individual animal was calculated based on the body weight measured most recently.

5. Preparation of dosing solutions

5.1 Preparation method:

Specified amounts of ^{14}C -DEHP and DEHP were dissolved in corn oil and fixed to a specified concentration by stirring and dissolving with a stirrer. The dosing solution was stored in a refrigerator and protected from light.

5.2 Determination of concentration:

The DEHP concentration in the dosing solution prepared in the above method has been confirmed in the toxicological study (SECTION-I), thus no measurement regarding concentration of DEHP was conducted and measured radioactivity concentration only.

5.3 Determination of stability:

After it was used, radiochemical purity of ^{14}C -DEHP was measured by the radio-HPLC method and confirmed that it was radiochemically stable at dosing. The stability regarding the DEHP concentration has been confirmed in the toxicological study (SECTION-I), thus it was not measured in the study.

6. Study design and sample collection

Study item	Dose level (mg/kg)	Number of animal (animal no.)						Ref.
		3 months old		18 months old				
		Group 3		Group 4		Group 5		
		Male	Female	Male	Female	Male	Female	
Plasma concentration and Excretion	100	3 (20201-20203)	3 (60201-60203)	3 (30201-30203)	3 (70201-70203)	3 (40201-40203)	3 (80201-80203)	6.1
	2500	3 (20401-20403)	3 (60401-60403)	3 (30401-30403)	3 (70401-70403)	3 (40401-40403)	3 (80401-80403)	
Tissue Distribution	100	3 (20201-20203)	3 (60201-60203)	3 (30202-30204)	3 (70201, 70202, 70204)	3 (40201-40203)	3 (80201-80203)	6.2
	2500	3 (20401-20403)	3 (60401-60403)	3 (30401-30403)	3 (70401-70403)	3 (40401-40403)	3 (80401-80403)	

- With an interval of more than 2-week after examination of parameters in Section 6.1, the animals were administered with ^{14}C -DEHP again and the parameters in Section 6.2 were examined. The same animal was used in Section 6.1 and 6.2 except animal No. 70204.
- The measurement of excretion rate into feces in Section 6.1 was examined only in males of the Groups 3 and 4.
- Group 3: administrated singly with ^{14}C -DEHP.
- Group 4: administrated singly with ^{14}C -DEHP. Animal Nos. 30201 was sacrificed and 70203 died at the end of the experiments in Section 6.1. For that reason, other animals designated as 30204 and 70204 were used for the experiments in Section 6.2.
- Group 5: Pretreated with DEHP for 65 weeks and then administrated singly with ^{14}C -DEHP. They were continuously administered with DEHP from the next day of ^{14}C -DEHP administration to the day before administration in Section 6.2. Since Animal No. 80202 died during the experiments in Section 6.1, parameters in Section 6.2 was not examined.

6.1 Determination of radioactivity concentration in plasma and excretion of radioactivity into urine and feces

6.1.1 Determination of radioactivity concentration in plasma:

Blood samples were collected from the femoral vein at the time points of 1, 2, 4, 8, 12, 24, 48, 72, 120, and 168 hours after administration. Blood collection volume was approximately set at 120 μL for the 3 months old animals and 250 μL for the 18 months old animals. The blood samples were heparinized (Shimizu Pharmaceutical Co., Ltd.), and then centrifuged (4°C, 11,000 min^{-1} , 5 minutes, HITACHI CF15D2) to obtain plasma sample. Radioactivity in a portion of each plasma sample (n=1) was measured in accordance with the method in Section 7 to determine the plasma radioactivity concentration. Plasma sample volume was set at 50 μL for the 3 months old animals and 100 μL for the 18 months old animals.

6.1.2 Determination of excretion of radioactivity into urine and feces:

Spontaneously excreted urine and feces were collected separately at the time points of 0-24, 24-48, 48-72, 72-120, and 120-168 hours after the administration, and radioactivity in these samples was measured in accordance with the method in Section 7. At each time point of urine collection, the bottom surface of the cage was washed with purified water.

The water used for the cage washing was mixed with the urine sample and the measured radioactivity was used as the urine sample.

6.2 Determination of radioactivity concentration in tissues

Animals received ^{14}C -DEHP at an interval of more than 2 weeks after previous treatment for 6.1, radioactivity concentrations in tissues were determined. According to the results obtained in Section 6.1, sampling time was decided to be 2-hour post dose as the nearest the maximum concentration time (t_{max}). The abdominal cavity of each animal was opened under anesthesia with pentobarbital, and blood was collected from the posterior vena cava. Then, the animals were euthanized by abdominal aorta exsanguination, and the following tissues were removed. The bile was collected from the gall bladder using a syringe with an injection needle. The sampled tissues were rinsed with saline, and water droplets remaining on the surface of the tissues were removed with a sheet of filter paper. The blood samples were heparinized, and 100 μL of each sample was designated as the blood sample ($n=1$), and the remaining portion of the sample was centrifuged (4°C , 1,600 g, 10 minutes, HITACHI CF7D2). The resulting supernatant was taken in the amount of 100 μL and designated as the plasma sample ($n=1$). These samples were measured for radioactivity as described in "7. Measurement of radioactivity" to determine the concentrations of radioactivity.

[Tissues for measurement of radioactivity]

- | | | | |
|----------------------------|--------------|------------|-------------------|
| - Blood | - Plasma | - Brain | - Pituitary |
| - Thyroid | - Heart | - Lung | - Liver |
| - Bile in the gall bladder | | - Adrenal | - Kidney |
| - Spleen | - Pancreas | - Fat | - Muscle femoral |
| - Testis | - Epididymis | - Prostate | - Seminal vesicle |
| - Ovary | - Uterus | - Carcass | |

7. Measurement of radioactivity

Radioactivity was measured by a liquid scintillation counter (LSC, TRI-CARB 2300TR, Packard) with the tSIE (transformed Spectral Index of External standard) method being used for quenching correction. Each sample was measured once for 5 minutes. A net count was determined by subtracting the background data (the liquid scintillation cocktail was measured once for 10 minutes) from observed original count. The detection limit of the radioactivity was prescribed to be 1.5-fold of the background data (cpm).

The following paragraphs describe the method of sample preparation for LSC measurement.

7.1 Dosing solution

50 μ L (n=3) of the dosing solution was collected and diluted to 10 mL with acetone. Each 1.0 mL (n=1) of the diluted solution was collected and mixed with a 5 mL of scintillation cocktail (Clearsol I, Nacalai Tesque).

7.2 Plasma

One milliliter of water was added to vials containing samples, and mixed with a 10 mL of Clearsol I.

7.3 Urine and bile in the gall bladder

The whole weight was measured for each sample. About 0.1 g of sample (n=2) was weighed, and mixed with a 10 mL of Clearsol I.

The remaining portions of the samples were stored frozen (at about -20°C).

7.4 Feces

After removing an alien substance such as feed, about 9-fold volume (v/w) of water was added to feces, whole weight was measured, and then a suspension was prepared with a polytron homogenizer. About 0.5 g each of suspension (n=2) was weighed, 1 mL of tissue solubilizer (Solubilizer of exclusive use for BIOMERIT, Nacalai Tesque) was added, and after that, it was treated by a tissue dissolving apparatus for 30 minutes (Daiichi Pure Chemicals). This was finally mixed with 15 mL of scintillation cocktail (HIONIC-FLUORTM, PACKARD).

The remaining portions of the suspension were stored frozen (at about -20°C).

7.5 Blood

One milliliter aliquot of tissue solubilizer was added, treated for 30 minutes by BIOMERIT, then mixed with a 15 mL of HIONIC-FLUORTM.

7.6 Tissues

After measuring the wet weight, the tissue was cut into smaller pieces using a dissection scissors. About 0.1 g of tissue (n=1) was weighed, 1 mL of tissue solubilizer was added. This was treated for 30 minutes by BIOMERIT, then mixed with 15 mL of HIONIC-FLUORTM.

The remaining portions of the cut tissue were stored frozen (at about -20°C).

7.7 Carcass

The carcass was supplemented with 500 mL of 0.5 mol/L aqueous solution of sodium hydroxide and 80 mL of toluene and dissolved by refluxing while heating for 12 hours for 3 months old animals and for 7 to 52 hours for 18 months old animals. After allowed to cool,

the solution was weighed and homogenized by a Polytron Homogenizer. About 0.5 g (n=2) of the homogenate were taken, weighed, and mixed with 15 mL of HIONIC-FLUOR™.

The remaining homogenates were discarded.

8. Data analysis

8.1 Calculation and expression of the study results

The data including the analytical result of radioactivity, plasma concentration, concentration in tissues and distribution rate, and cumulative excretion rate were processed on a pharmacokinetic study-supporting system ADME SUPPORT Ver. 1 (FFCSYSTEMS LIMITED). The individual radioactivity concentrations in the plasma were displayed in Appendix 1 to the decimal place (100-mg/kg group: to the second decimal place, 2500-mg/kg group: to the whole number) where the last digit was equivalent to the radioactivity detection limit that is expressed in DEHP concentration (μg equivalent of DEHP/mL or g). The individual results of radioactivity concentrations in the blood and tissues were displayed in the Appendix to the same decimal place as the plasma. Regarding the individual results of the cumulative excretion rate and distribution rate, as the percentages to the administered radioactivity, displayed in the Appendix to the second decimal place and to the third decimal place, respectively. The ratio of each radioactivity level in tissues to the plasma concentration was calculated based on the indicated mean value and indicated in the Table to the third decimal place. When an individual out of 3 animals resulted in N.D. in certain tissue(s), calculations were made assuming as n=3 and assigning 0 to N.D.'s: when 2 or more animals resulted in N.D., a result of N.D. was displayed.

8.2 Pharmacokinetic analysis

The profile of the radioactivity concentrations in plasma was analyzed using an pharmacokinetic analysis software Win Nonlin Ver.3.1 (Pharsight Corporation), and various pharmacokinetic parameters (AUC, etc) were calculated. Measured values were employed for the C_{max} , and t_{max} . C_{max} , was defined as the highest concentration observed and t_{max} was the measurement time when it was observed.

The $t_{1/2}$ was defined as the scheduled measurement time when the concentration went less than $1/2 C_{\text{max}}$ for the first time. AUC_{all} was calculated based on the measured values obtained assuming final scheduled measurement as 0 (zero) when all the scheduled measurement were valid. When ND measurement appeared, the first ND was assumed 0 (zero) and all the measurement time point prior to the first ND were brought into account. $AUC_{\text{all}}/\text{Dose}$ ratio value was obtained by dividing it by dosing volume.

Results

1. *Radioactivity Concentration in Plasma*

Individual pharmacokinetic parameters are shown in Table 2. Animals of following number did not show a significant elevation of the concentration in blood /plasma. The interpretation was described in the discussion section.

- #20201 (100 mg/kg-male, 3 months old)
- #20402 (2500 mg/kg-male, 3 months old)
- #40201 (100 mg/kg-male, 18 months old)
- #70202 (100 mg/kg-female, 18 months old)
- #80401 (2500 mg/kg-female, 18 months old)
- #80402 (2500 mg/kg-female, 18 months old)
- #80403 (2500 mg/kg-female, 18 months old)

1.1 Juvenile animals (Group 3)

The plasma radioactivity concentrations of ^{14}C -DEHP in 3 months old common marmosets after a single oral administration of 100 or 2500 mg/kg are displayed in Table 1-1 and Figure 1-1. Pharmacokinetic parameters are shown in Table 2-1.

1.1.1 100-mg/kg administration group

Individual data is shown in Appendix 1-1 for male.

Animal #20201 is included.

Individual data is shown in Appendix 1-2 for female.

1.1.2 2500-mg/kg administration group

Individual data is shown in Appendix 1-3 for male.

Animal #20402 is included.

Individual data is shown in Appendix 1-4 for female.

1.2 Adult animals (Group 4)

The plasma radioactivity concentrations of ^{14}C -DEHP in 18 months old common marmosets after a single oral administration of 100 or 2500 mg/kg are displayed in Table 1-2 and Figure 1-2. Pharmacokinetic parameters are shown in Table 2-2.

1.2.1 100-mg/kg administration group

Individual data is shown in Appendix 1-5 for male.

Individual data is shown in Appendix 1-6 for female.

Animal #70202 is included.

1.2.2 2500-mg/kg administration group

Individual data is shown in Appendix 1-7 for male.

Individual data is shown in Appendix 1-8 for female.

1.3 Adult animals with pretreatment of DEHP (Group 5)

1.3 Adult animals with pretreatment of DEHP (Group 5)

The plasma concentrations of ^{14}C -DEHP in 18 months old common marmosets, which had been pretreated with repeated oral doses of DEHP for 65 weeks, after a single oral administration of 100 or 2500 mg/kg are displayed in Table 1-3 and Figure 1-3. Pharmacokinetic parameters are shown in Table 2-3.

1.3.1 100-mg/kg administration group

Individual data is shown in Appendix 1-9 for male.

Animal # 40201 is included.

Individual data is shown in Appendix 1-10 for female.

1.3.2 2500-mg/kg administration group

Individual data is shown in Appendix 1-11 for male.

Individual data is shown in Appendix 1-12 for female.

All the three animals (#80401, #80402, #80403) did not show a significant elevation of the concentration in blood /plasma.

When a single dose of ^{14}C -DEHP was administered orally to common marmosets that had been pretreated with repeated oral doses of DEHP for 65 weeks, t_{max} was 1-4 hours – similar to the group that was not pretreated. However, C_{max} and AUC values for females in the pretreated group were lower than the males – a result opposite from the non-pretreated group – which indicates a change in the metabolism of DEHP through multiple doses.

2. *Radioactivity Concentration in Urine and Feces*

2.1 Juvenile animals (Group 3)

The radioactivity concentrations in urine and feces of ^{14}C -DEHP in 3 months old male common marmosets after a single oral administration of 100 or 2500 mg/kg are displayed in Table 3-1 and Figures 2-1 and 2-2.

2.1.1 100-mg/kg administration group

The level of radiation excreted in urine and feces up until 72 hours after administration

was 16.1% and 34.7% of dosage radiation, respectively. Excretion of radiation after 72 hours was slight, reaching a cumulative excretion rate of 18.3% and 35.8% in urine and feces respectively up until 168 hours, for a total excretion rate of 54.1%.

2.1.2 2500-mg/kg administration group

The level of radiation excreted in urine and feces up until 72 hours after administration was 8.4% and 58.9% of dosage radiation respectively. As with 100-mg/kg group, excretion of radiation after 72 hours was slight, with cumulative excretion reaching 9.9% and 60.8% in urine and feces respectively up until 168 hours, for a total excretion rate of 70.7%.

2.2 Adult animals (Group 4)

The radioactivity concentrations in urine and feces of ^{14}C -DEHP in 18 months old male common marmosets after a single oral administration of 100 or 2500 mg/kg are displayed in Table 3-2 and Figures 2-3 and 2-4.

2.2.1 100-mg/kg administration group

The level of radiation excreted in urine and feces up until 72 hours after administration was 12.0% and 62.7% of dosage radiation, respectively. Excretion of radiation after 72 hours was slight, with cumulative excretion reaching 12.8% and 65.6% in urine and feces respectively up until 168 hours, for a total excretion rate of 78.4%.

2.2.2 2500-mg/kg administration group

The level of radiation excreted in urine and feces up until 72 hours after administration was 20.7% and 44.0% of dosage radiation, respectively. Excretion of radiation after 72 hours was slight, with cumulative excretion reaching 22.2% and 46.7% in urine and feces respectively up until 168 hours, for a total excretion rate of 68.8%.

Animal #30403 (see Appendix 2-4) experienced severe watery feces diarrhea during the first 24 hours of excreta. For more strict interpretation, data from the animal might be carefully considered.

3. Radioactivity Concentration in Tissue

3.1 Juvenile animals (Group 3)

The radioactivity concentrations in tissues of ^{14}C -DEHP in 3 months old common marmosets 2 hours after a single oral administration of 100 or 2500 mg/kg are displayed in Table 4-1 and Figures 3-1 and 3-2. Distribution rates of radioactivity in tissue are shown in Table 5-1. Individual data are shown in Appendices 3-1, 3-2, 4-1 and 4-2.

3.1.1 100-mg/kg administration group

[Male] Radioactivity was highest in the kidneys (52.82 $\mu\text{g eq./g}$), with the mean value for 3 animals 1.99 times higher than the mean for plasma concentration (26.54 $\mu\text{g eq./mL}$). Radioactivity in the male reproductive organs was 5.47 $\mu\text{g eq./g}$ in the testes, 10.25 $\mu\text{g eq./g}$ in the epididymis, concentrations which are equivalent to 21% and 39% of plasma, respectively. Distribution rates of radioactivity were highest in the liver and kidneys at 0.691% and 0.372% of the dose respectively. Concentration in bile in the gall bladder was 805.73 $\mu\text{g eq./g}$, or a distribution rate of 0.391% of the dose. In individual, an animal (# 20201) exhibited higher radiation in the prostate and seminal vesicle than in plasma. The cause is, however, attributable to the contamination with urine, despite careful sampling.

[Female] Radioactivity was highest at the kidneys (67.65 $\mu\text{g eq./g}$) also in female common marmosets, at a concentration greater than plasma (33.55 $\mu\text{g eq./mL}$) by a factor of 2.02. However, all other tissue types, including the reproductive organs, displayed a lower radioactivity concentration than the plasma. Distribution rates of radioactivity were highest in the liver and kidneys at 0.792% and 0.442% of the dose, respectively. Concentration in bile in the gall bladder was 1325.84 $\mu\text{g eq./g}$, or a distribution rate of 0.776%

3.1.2 2500-mg/kg administration group

[Male] As with the 100-mg/kg administration group was highest in the kidneys of males (84.23 $\mu\text{g eq./g}$), equivalent to 1.85 times the concentration in plasma (45.51 $\mu\text{g eq./mL}$). Also, as with the 100-mg/kg group, although one animal was identified as having higher radioactivity levels in the prostate and seminal vesicle than in the plasma, mean radioactivity values for all tissues except kidneys were less than for plasma. Radioactivity in the male reproductive organs was 10.52 $\mu\text{g eq./g}$ in the testes, 14.09 $\mu\text{g eq./g}$ in the epididymis, 34.22 $\mu\text{g eq./g}$ in the prostate, and 23.14 $\mu\text{g eq./g}$ in the seminal vesicle – concentrations which are equivalent to 23%, 31%, 75%, and 51% of plasma, respectively. Distribution rates of radioactivity were highest in the liver and kidneys at 0.054% and 0.027% of the dose, respectively. Concentration in bile in the gall bladder was 1341.18 $\mu\text{g eq./g}$, or a distribution rate of 0.051% of the dose. In individual, an animal (# 20403) was identified as having higher radioactivity levels in the prostate and seminal vesicle than in the plasma, The cause is, however, attributable to the contamination with urine, despite careful sampling.

[Female] Radioactivity was also highest in the kidneys (92.32 $\mu\text{g eq./g}$) in female common marmosets, at a concentration higher than plasma (45.50 $\mu\text{g eq./mL}$) by a factor of 2.03. However, all other tissue types, including the reproductive organs, displayed a lower radioactivity concentration than the plasma. Distribution rates of radioactivity were highest in the liver and kidneys at 0.054 and 0.031% of the dose, respectively. Concentration in bile in the gall bladder was 1440.27 $\mu\text{g eq./g}$, or a distribution rate of 0.044% of the dose. In individual, an animal (# 60401) exhibited relatively low blood concentration. High (>1) tissue / plasma concentration ratio for fat was noticed.

3.2 Adult animals (Group 4)

The radioactivity concentrations in tissues of ^{14}C -DEHP in 18 months old common marmosets 2 hours after a single oral administration of 100 or 2500 mg/kg are displayed in Table 4-2 and Figures 3-3 and 3-4. Distribution rates of radioactivity in tissue are shown in Table 5-2. Individual data are shown in Appendices 3-3, 3-4, 4-3, and 4-4.

3.2.1 100-mg/kg administration group

[Male] In male common marmosets, radioactivity concentration in the kidneys (28.49 $\mu\text{g eq./g}$) and seminal vesicle (16.26 $\mu\text{g eq./g}$) was found to exceed that of plasma (9.01 $\mu\text{g eq./mL}$) by a factor of 3.16 and 1.80, respectively. The concentration in the seminal vesicle was the result of considerable individual variation. All other tissues exhibited a similar or lower level of concentration than the plasma. Radioactivity in the testes (0.83 $\mu\text{g eq./g}$), epididymis (1.97 $\mu\text{g eq./g}$), and prostate (4.59 $\mu\text{g eq./g}$) were equivalent to 9, 22, and 51% of plasma, respectively. Distribution rates of radioactivity were highest in the liver and kidneys at 0.266% and 0.180% of the dose, respectively. Concentration in bile in the gall bladder was 383.69 $\mu\text{g eq./g}$, or a distribution rate of 0.176% of the dose. In individuals, an animal (# 30202) exhibited higher radiation in the prostate and seminal vesicle than in plasma. The cause is, however, attributable to the contamination with urine, despite careful sampling. An animal (# 30203) exhibited a relatively low plasma concentration. High (>1) tissue / plasma concentration ratio for fat, liver or epididymis was noticed. Epididymis is technically less plausible to be contaminated by urine and considered invalid.

[Female] Radioactivity was also higher in the kidneys (55.62 $\mu\text{g eq./g}$) in female common marmosets than plasma radioactivity (16.88 $\mu\text{g eq./mL}$) by a factor of 3.30. However, all other tissue types, including the reproductive organs, displayed a lower radioactivity concentration than the plasma. Distribution rates of radioactivity were highest in the liver and kidneys at 0.426% and 0.364% of the dose, respectively. Concentration in bile in the gall bladder was 571.55 $\mu\text{g eq./g}$, or a distribution rate of 0.353% of the dose.

3.2.2 2500-mg/kg administration group

[Male] Radioactivity was highest in male common marmosets in the kidneys (110.38 $\mu\text{g eq./g}$), equivalent to 1.69 times the concentration was found to exceed that of plasma (65.48 $\mu\text{g eq./mL}$). However, all other tissue types, including the reproductive organs, displayed a lower radioactivity concentration than plasma. Radioactivity in the male reproductive organs was 8.47 $\mu\text{g eq./g}$ in the testes, 15.98 $\mu\text{g eq./g}$ in the epididymis, 10.64 $\mu\text{g eq./g}$ in the prostate, and 13.84 $\mu\text{g eq./g}$ in the seminal vesicle, concentrations which are equivalent to 13%, 24%, 16%, and 21% of plasma, respectively. Distribution rates of radioactivity were highest in the liver and kidneys at 0.073% and 0.036% of the dose, respectively. Concentration in bile in the gall bladder was 2172.04 $\mu\text{g eq./g}$, or a distribution rate of 0.034% of the dose.

[Female] Radioactivity was also higher in the kidneys (226.02 $\mu\text{g eq./g}$) in female common marmosets than that in plasma (123.74 $\mu\text{g eq./mL}$) by a factor of 1.83. However, all other tissue types, including the reproductive organs, displayed a lower radioactivity concentration than plasma. Distribution rates of radioactivity were highest in the liver and kidneys at 0.102% and 0.054% of the dose, respectively. Concentration in bile in the gall bladder was 2865.25 $\mu\text{g eq./g}$, or a distribution rate of 0.060% of the dose.

3.3 Adult animals with pretreatment of DEHP (Group 5)

The concentrations of ^{14}C -DEHP in tissues of 18 months old common marmosets, which had been pretreated with repeated oral doses of DEHP for 65 weeks, 2 hours after a single oral administration of 100 or 2500 mg/kg are displayed in Table 4-3 and Figures 3-5 and 3-6. Distribution rates of radioactivity in tissue are shown in Table 5-3. Individual data are shown in Appendices 3-5, 3-6, 4-5 and 4-6.

3.3.1 100-mg/kg administration group

[Male] In male common marmosets, radioactivity in the kidneys (85.55 $\mu\text{g eq./g}$) was found to exceed levels in plasma (29.92 $\mu\text{g eq./mL}$) by a factor of 2.86. Radioactivity concentrations in all other tissues were lower than in the plasma. Radioactivity in the male reproductive organs was 3.03 $\mu\text{g eq./g}$ in the testes, 9.79 $\mu\text{g eq./g}$ in the epididymis, 7.34 $\mu\text{g eq./g}$ in the prostate, and 12.60 $\mu\text{g eq./g}$ in the seminal vesicle, concentrations which are equivalent to 10%, 33%, 25%, and 42% of plasma respectively. Distribution rates of radioactivity were highest in the liver and kidneys at 1.061% and 0.497% of the dose, respectively. Concentration in bile in the gall bladder was 1048.92 $\mu\text{g eq./g}$, or a distribution rate of 0.411% of the dose.

[Female] Radioactivity concentration was highest in the kidneys (105.34 $\mu\text{g eq./g}$) in female common marmosets, exceeding concentrations in plasma (47.28 $\mu\text{g eq./mL}$) by a factor of 2.23. However, all other tissue types, including reproductive organs, displayed a lower radioactivity concentration than in the plasma. Distribution rates of radioactivity were high in the liver and kidneys at 1.084% and 0.697% of the dose, respectively. Concentration in bile in the gall bladder was 662.73 $\mu\text{g eq./g}$, or a distribution rate of 0.411% of the dose.

3.3.2 2500-mg/kg administration group

[Male] Radioactivity was highest in male common marmosets in the kidneys (223.94 $\mu\text{g eq./g}$), exceeding concentrations in plasma (102.78 $\mu\text{g eq./mL}$) by a factor of 2.18. Radioactivity concentrations in all other tissues were lower than in plasma. Radioactivity in the male reproductive organs was 12.40 $\mu\text{g eq./g}$ in the testes, 27.98 $\mu\text{g eq./g}$ in the epididymis, 20.38 $\mu\text{g eq./g}$ in the prostate, and 23.73 $\mu\text{g eq./g}$ in the seminal vesicle, concentrations which are equivalent to 12%, 27%, 20%, and 23% of plasma, respectively. Distribution rates of radioactivity were high in the liver and kidneys at 0.121% and 0.068%

of the dose, respectively. Concentration in bile in the gall bladder was 2507.74 $\mu\text{g eq./g}$, or a distribution rate of 0.068% of the dose.

[Female] Radiation was also highest in the kidneys (97.74 $\mu\text{g eq./g}$) in female common marmosets, exceeding concentrations in plasma radioactivity (41.70 $\mu\text{g eq./mL}$) by a factor of 2.34. However, all other tissue types, including the reproductive organs, displayed a lower radioactivity concentration than in the plasma. All tissues, except brain, muscle, or fat, displayed the same level of radioactivity concentrations (about 20-60% of plasma) found in males. Distribution rates of radioactivity were highest in the liver and kidneys at 0.054% and 0.030% of the dose, respectively. Concentration in bile in the gall bladder was 1500.20 $\mu\text{g eq./g}$, or a distribution rate of 0.027% of the dose. In individual, an animal (# 80401) showed high (>1) tissue / plasma concentration ratio for fat, or muscle femoral.

DISCUSSION AND CONCLUSION

<Repeated dose toxicological study>

DEHP was administered by oral gavage at doses of 0, 100, 500, and 2500 mg/kg for 65 weeks to common marmosets of both sexes, and its toxicity was assessed. The treatment started as early in the life stage (about 3 months old) as technically possible and continued until sexual maturation (about 18 months old). However, several animals succumbed during the treatment period to wasting syndrome that is considered usual in this species of out-bred experimental animal. The wasting animals that showed marked weight reduction due to loose feces and diarrhea appeared sporadically in the early stage of administration in non-dose-dependent way. Therefore, the occurrence was considered to be incidental and not to be treatment-related, and such wasting animals were omitted from the toxicity assessment.

Treatment-related change was not evident in the clinical sign, body weight, hematology, or blood chemistry. No change relating to the treatment was observed in organ weight or macro/H.E.-microscopic findings of the non-reproductive organs. Ovary weight increased in dose-dependent manner. However, such an ovary was similar to that of more matured females (3 – 4 years old or more) and showed no histological abnormality.

Concerning the testis, no treatment-related change was observed. During the treatment period, all males experienced testosterone surge, and the testosterone levels in all treated groups were similar to that of the control group. For the testis, electron microscopic examination was additionally applied, however, this revealed no treatment-related abnormality. Histochemical examination after 3β -hydroxysteroid dehydrogenase (3β -HSD) staining did not reveal any alteration in steroid synthesis. Consumption of peroxide scavenger such as GSH, GST, or GSH-Px in the testis was not noticed, which suggests that peroxisomal enzyme may not be operating in this organ. For functional examination, sperm count was conducted to show no treatment related effect in numerical changes.

Concerning the liver, following adaptive changes to the test-substance were observed. The liver weight and its body weight ratio were not affected. Peroxisomal enzymes such as CAT, CPT, and FAOS showed no increases in their activities. P450 content, however, tended to increase with dose-dependent manner in general. With regard to specific CYPs, CYP3A and 2E that are related to testosterone 6β hydration and lauric acid $\omega-1$ hydration, respectively and CYP4A that is related to lauric acid ω hydration, showed no marked or specific increase. These facts showed induction of non-PP dependent oxidase and proved absorption of the test substance.

In the toxicological study, despite the high dose of 2500mg/kg and adaptive liver change proving absorption of DEHP, no testicular change was morphologically or functionally noticed in the extensive examinations.

<Pharmacokinetic study>

Pharmacokinetic parameters such as blood levels, excretions in the urine and feces, or tissue distributions were examined using a ring-labeled DEHP in juvenile (about 3 months old) and adult (about 18 months old, with or without DEHP-pretreatment) animals when ^{14}C -DEHP was administered singly.

Radioactivity Concentration in Plasma: Animals of following number did not show significant elevation of the concentration in blood /plasma: #20201 (100 mg/kg-male), #70202 (100mg/kg -female), #40201 (100mg/kg -male), #20402 (2500 mg/kg -male), #80401 (2500 mg/kg -female), #80402 (2500 mg/kg -female), and #80403 (2500 mg/kg -female). The male animals among them were subjected to the measurement for excretion in the urine /feces. They revealed substantial urine excretion. It could be assumed that absorption of DEHP are normal in these animals and the reason for the lack of significant elevation in blood /plasma concentration may be attributable to rapid metabolism and/or excretion. The assumption may be supported by the observation that all the females that have experienced prior treatment with high dose (2500 mg /kg/day for 65weeks, #80401, #80402, and #80403) are most likely to have acquired metabolic activation and did not show the elevation.

Because of variability among individuals, average value of t_{max} from animals in group 3 (male three-month old common marmosets in the 2500 mg/kg administration including #20402) was 10.0 hours; however, when mean concentration values at each measurement time point were used to calculate t_{max} , the result was 2 hours. The mean t_{max} value for all other groups were in the 1 – 4-hour range, suggesting that DEHP was absorbed in the digestive tract rapidly after oral administration. After t_{max} , plasma radioactivity levels decreased in a two-phase decay. The AUC_{all} /dose ratio was 0.374 – 1.508 for the 100-mg/kg administration group and 0.108-0.381 for the 2500-mg/kg administration group, except pretreated females, indicating a drop in absorption ratio accompanying higher dosage levels. The blood / plasma concentration decreased to less than half the C_{max} by 8 hours after administration except for # 20201, #20402 and 80201.

Excretion Rates in Urine and Feces: In both 3 and 18 months old animals, the primary excretion route for ^{14}C -DEHP after a single dose was feces. About 10% to 22% of administered radioactivity was also excreted in the urine. Even at the high dosage of 2500 mg/kg, at least 10% of the radioactivity was absorbed in the digestive tract. Radioactivity was also detected in bile in the gall bladder, which suggests the existence of excretion through bile. A portion of the radioactivity excreted through feces is thought to have originated in the bile. Therefore, the actual digestive tract absorption rate is thought to be

greater than when calculated from the urine excretion rate.

The total urine and feces radioactivity excretion rate up to 168 hours was 54% to 78%. In the present study, the excretion test was conducted simultaneously with measurement of changes in plasma concentration over time. It was therefore considered possible that a portion of the radiation was not recovered because of urination during blood collection. Further, the proportion of residual radiation in the body after 168 hours was not measured, so it is considered that a portion of the non-recovered radiation remained in the bodies.

Radioactivity Concentration in Tissue: Two hours after a single oral administration of ^{14}C -DEHP, all groups displayed the highest level of radiation in their kidneys, which is thought to be a result of urine excretion. In some animals, the level of radiation detected in the prostate and seminal vesicle was greater than that detected in plasma, but the evident variation in individuals leads us to consider contamination from radioactivity in urine as the cause of this phenomenon. Including these individuals, there was no group in which the mean level of radioactivity concentration detected in any tissue, except for the kidneys, exceeded that measured in plasma. No abnormal distribution of radioactivity was observed in the testes or other male reproductive organs, for which toxicity in rodents has been reported. The same was true for female reproductive organs such as the ovaries and uterus.

Distribution trends remained unchanged when a single dose of ^{14}C -DEHP was administered orally to common marmosets that had been pretreated with repeated oral doses of DEHP for 65 weeks, suggesting that repeated administrations of DEHP did not affect bio-distribution.

The above results show that a single oral dose of DEHP leads to at 10% or greater absorption in the digestive tract that account for only urine excretion. When extensive excretion to bile observed is taken into the consideration, the figure should be added with bile excretion. Quantitative estimation of the extent of bile excretion is difficult. The extent was, however, considered substantial, because bile excretion was so significant that re-elevation of plasma concentration was observed in some cases that may have caused by re-absorption through entero-hepatic circulation. As an inference to toxicological finding in SECTION 1 from the established absorption, the lack of the effect on the testes in common marmoset, which has been observed in rodents after repeated DEHP administration. Therefore, it can no longer be assumed that this is due to poor absorption. This difference is thought to arise from a difference in target organs physiology between the 2 animal species rather than from any significant differences in metabolic kinetics. It should be stated that radioactivity in testes showed about 1/10 as much as of the blood radioactivity concentration. Meaningful accumulation in testes was not observed during distribution measurement for various tissues and organs. The result is consistent with the lack of testicular effect in any observation including EM histopathology conducted in oral dose toxicity study in juvenile common marmosets described in SECTION-F'.

As a conclusion, when DEHP administered orally to juvenile marmoset at dosage levels

of 100, 500 and 2500 mg/kg/day for 65 weeks until maturation, testicular effect that is well known in rodents were not observed despite extensive examination. The apparent mechanism of the species deference may be related to the evidence of limited accumulation of the metabolite in testes.

STUDY NOTES

1. Unforeseeable events which may have had an effect on the reliability of the study

(1) The total excretion rate for feces and urine never exceeded the level of 54% to 78% up to 168 hours after administration. This measurement was conducted by performing the excretion test and the measurements for changes in plasma concentration over time simultaneously. It was therefore considered possible that a portion of the radio-activity was not recovered because of urination during blood collection. Further, the proportion of residual radiation in the body after 168 hours was not measured, so it is considered that a portion of the unaccounted for radiation remained in the bodies.

(2) An animal (#10213) which was assigned to group 1 was initially sexed to wrong gender and found to be female at the necropsy. All the pertinent data were not included in the report.

(3) An animal (#80202) died during experiment to determine plasma or excreta, following treatment and determination of tissue distribution was not performed on it.

2. Deviations from the protocol

(1) Dosing preparation storage temperature exceeded permissible range (2.0° - 15.0°C). Records read (1.5° - 16.0°C). Time while exceeded is transient.

(2) Animals were observed to undergo a general decline in health when they were transferred to a RI-controlled area prior to test substance administration. This was thought to be a result of the change in environment. Various actions, such as supplementing their feed, were attempted, but some animals died. As a result, other animals were substituted (for details, see Section 6. Since the substituted animals had undergone the same care in the same conditions, this was thought to have no influence on the study.

(3) An animal (#80202) died after test substance administration. Necropsy findings revealed death to be the result of physical damage to the thoracic and tracheal regions incurred during administration. Since at this point no animal could be substituted, results for this animals group were obtained by the mean values obtained from the remaining 2 animals.

(4) Number of 74 male and 56 female animals purchased was instead of 54 males or 42 females written in the protocol. Some animals, however, were succumbed and replaced as described in SECTION-I and II item 6.

(5) Clinical sign and body weight for animals in group 5 was measured. Rationale: Need for determination of the compound dosage.

- (6) Epididymis from animals in group 2 were examined histopathologically. Rationale: To examine the sperm contained by means of comparable method.
- (7) Administration periods of some animals were extended up to 5 days because of holiday or tentative and urgent non-availability of study director.
- (8) During pharmacokinetic study (SECTION-II), room temperature and humidity exceeded permissible range (23.0° – 30.8°C, RH 35.0%-75.0%). Records read (22.3° C – 30.8°C) and (26.0% – 60.2%). Duration while exceeded is transient.
- (9) During pharmacokinetic study (SECTION-II), room lighting made on when examination (e.g. blood or urine sampling) or animal care. Rationale: Being necessary process for experiment.
- (10) Plasma sampling for radioactivity measurement was performed up to 168 hours after dosing. Rationale: Plasma content of radioactivity at 24 hours was still meaningfully detected, showing the need for extension of sampling time.

All the deviations listed above are considered not to inflict on the scientific integrity of the study.

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Quality Assurance Statement

Sponsor: Japan Plasticizer Industry Association
 Title: Sixty-Five-Week Repeated Oral Dose Toxicity Study of
 Di(2-ethylhexyl)phthalate (DEHP) in Juvenile Common Marmosets
 Study No.: B000496

This study was carried out in accordance with the following standard. I hereby certify that this final report faithfully describes the methods and results in this study. The phase and dates of inspection and reporting are as follows.

OECD Principles of Good Laboratory Practice (as revised in 1997)
 Japanese GLP on Industrial Chemicals (1984, 1988, 2000)

Phase	Inspector	Inspection Date	Reporting Date	
			to the Study Director	to the Management
Study Protocol	Akiko Esaki	Aug. 06, 2000	Aug. 06, 2000	Aug. 06, 2000
Protocol Amendment	Akiko Esaki	Aug. 28, 2000	Aug. 28, 2000	Aug. 28, 2000
No.1	Akiko Esaki	Sep. 20, 2000	Sep. 20, 2000	Sep. 20, 2000
No.2	Akiko Esaki	Sep. 25, 2000	Sep. 25, 2000	Sep. 25, 2000
No.3	Akiko Esaki	Sep. 25, 2000	Sep. 25, 2000	Sep. 25, 2000
No.4	Akiko Esaki	Oct. 16, 2000	Oct. 16, 2000	Oct. 16, 2000
No.5	Akiko Esaki	Oct. 28, 2000	Oct. 28, 2000	Oct. 28, 2000
No.6	Akiko Esaki	Nov. 22, 2000	Nov. 22, 2000	Nov. 22, 2000
No.7	Akiko Esaki	Dec. 22, 2000	Dec. 22, 2000	Dec. 22, 2000
No.8	Akiko Esaki	Jan. 16, 2001	Jan. 16, 2001	Jan. 16, 2001
No.9	Akiko Esaki	Feb. 05, 2001	Feb. 05, 2001	Feb. 05, 2001
No.10 Draft	Akiko Esaki	Feb. 05, 2001	Feb. 05, 2001	Feb. 05, 2001
No.10 Final	Akiko Esaki	Feb. 16, 2001	Feb. 16, 2001	Feb. 16, 2001
No.11 Draft	Akiko Esaki	Feb. 16, 2001	Feb. 16, 2001	Feb. 16, 2001
No.11 Final	Akiko Esaki	Feb. 21, 2001	Feb. 21, 2001	Feb. 21, 2001
No.12 Draft	Akiko Esaki	Feb. 21, 2001	Feb. 21, 2001	Feb. 21, 2001
No.12 Final	Akiko Esaki	Mar. 22, 2001	Mar. 22, 2001	Mar. 22, 2001
No.13 Draft	Akiko Esaki	Mar. 22, 2001	Mar. 22, 2001	Mar. 22, 2001
No.13 Final	Akiko Esaki	Dec. 25, 2001	Dec. 25, 2001	Dec. 25, 2001
No.14 Draft	Akiko Esaki	Dec. 25, 2001	Dec. 25, 2001	Dec. 25, 2001
No.14 Final	Akiko Esaki	Jun. 10, 2002	Jun. 10, 2002	Jun. 10, 2002
No.15 Draft	Akiko Esaki	Jun. 19, 2002	Jun. 19, 2002	Jun. 19, 2002
No.15 Final	Akiko Esaki			

Phase	Inspector	Inspection Date	Reporting Date	
			to the Study Director	to the Management
Study Procedure				
Receipt of animals	Akiko Esaki	Aug. 29, 2000	Aug. 29, 2000	Aug. 30, 2000
Preparation and analysis of dosing solution	Akiko Esaki	Sep. 26, 2000	Sep. 26, 2000	Sep. 29, 2000
Administration, body weight measurement	Akiko Esaki	Nov. 09, 2000	Nov. 09, 2000	Nov. 13, 2000
Administration, body weight measurement	Akiko Esaki	Dec. 12, 2000	Dec. 13, 2000	Dec. 14, 2000
Blood collection for hormonal analysis	Akiko Esaki	Jun. 28, 2001	Jun. 28, 2001	Jun. 28, 2001
Necropsy	Junko Wataji	Jan. 23, 2002	Jan. 24, 2002	Jan. 24, 2002
Raw Data, Draft Report				
	Akiko Esaki	Mar. 03, 2003	Mar. 17, 2003	Mar. 17, 2003
(Reinspection)	(Akiko Esaki)	-Mar. 14, 2003	(Mar. 17, 2003)	(Mar. 17, 2003)
Final Report	Akiko Esaki	Mar. 17, 2003	Mar. 17, 2003	Mar. 17, 2003
<B001107>				
Study Protocol				
Draft	Junko Wataji	Nov. 21, 2001	Nov. 21, 2001	Nov. 21, 2001
Final	Junko Wataji	Nov. 28, 2001	Nov. 28, 2001	Nov. 28, 2001
Protocol Amendment				
No.1 Draft	Junko Wataji	Dec. 20, 2001	Dec. 20, 2001	Dec. 21, 2001
No.1 Final	Junko Wataji	Dec. 21, 2001	Dec. 21, 2001	Dec. 21, 2001
Study Procedure				
Preparation of dosing solution	Junko Wataji	Dec. 07, 2001	Dec. 10, 2001	Dec. 10, 2001
Administration, collection of plasma	Junko Wataji	Dec. 11, 2001	Dec. 11, 2001	Dec. 11, 2001
Collection and preparation of excreta samples	Junko Wataji	Dec. 12, 2001	Dec. 12, 2001	Dec. 12, 2001
Collection of tissue samples	Junko Wataji	Dec. 25, 2001	Dec. 26, 2001	Dec. 26, 2001
Preparation of tissue samples	Junko Wataji	Dec. 26, 2001	Dec. 26, 2001	Dec. 26, 2001
Raw Data, Draft Report				
	Akiko Esaki	Mar. 12, 2003	Mar. 14, 2003	Mar. 17, 2003
(Reinspection)	(Akiko Esaki)	-Mar. 14, 2003	(Mar. 17, 2003)	(Mar. 17, 2003)
Final Report	Akiko Esaki	Mar. 17, 2003	Mar. 17, 2003	Mar. 17, 2003

Quality Assurance Manager: Tamotsu Nishitomi Date: March 17, 2003

Tamotsu Nishitomi

Quality Assurance Office, Kashima Laboratory
Mitsubishi Chemical Safety Institute Ltd.

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Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
Table 1
DEHP
Male (Group 1 and 2)
Body Weight

Dose (mg/kg)	Day	1	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147
0	mean	131.4	133.6	135.8	142.4	148.8	153.8	157.8	163.5	165.3	166.8	168.4	177.5	181.9	188.4	190.6	194.8	197.8	198.8	206.4	210.0	208.3	216.8
	S.D.	23.0	21.7	22.9	22.5	25.8	24.6	23.3	24.1	25.5	29.6	27.5	26.3	27.2	25.2	28.3	27.8	22.5	20.5	20.0	19.2	22.8	23.2
	n	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
100	mean	138.8	142.9	150.3	150.1	155.8	161.7	160.3	164.8	167.2	171.7	175.8	180.1	181.6	182.6	188.6	189.9	187.6	189.1	191.6	194.0	199.6	193.9
	S.D.	24.1	28.0	28.1	27.2	31.3	31.3	29.0	32.0	35.9	35.8	39.5	39.6	41.0	38.9	42.1	37.2	39.5	36.9	37.6	38.1	39.1	32.6
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
500	mean	132.5	137.0	144.6	145.2	148.0	151.8	156.5	160.1	165.3	168.5	172.3	175.6	182.1	181.9	182.5	185.8	192.7	195.2	199.6	203.4	202.6	206.6
	S.D.	21.1	20.7	20.6	20.6	23.5	22.7	23.1	22.0	23.2	23.8	25.4	27.5	27.7	32.3	29.2	33.3	33.7	33.9	39.5	40.4	41.7	42.9
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2500	mean	138.4	140.1	146.1	149.9	153.2	156.3	159.3	163.8	165.7	169.4	174.0	175.7	178.4	178.9	182.8	187.7	192.6	193.8	194.3	199.2	203.6	204.1
	S.D.	15.5	17.8	16.9	17.3	17.7	19.6	19.2	20.0	22.3	24.6	25.9	25.6	24.7	26.2	26.4	28.7	29.8	30.3	30.4	31.3	31.1	30.4
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9

Dose (mg/kg)	Day	154	161	168	175	182	189	196	203	210	217	224	231	238	245	252	259	266	273	280	287	294	301
0	mean	222.5	228	224.8	231.4	234.4	238.6	238.9	242.5	239.8	246.3	250.4	247.4	245.6	252.9	255.3	250.3	254.4	259	254.6	259.4	262	268.1
	S.D.	20.7	19.0	17.6	14.4	16.4	14.9	16.2	14.5	19.1	11.4	16.5	14.8	15.4	16.0	17.3	16.8	15.4	13.7	13.7	12.8	14.1	12.7
	n	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
100	mean	201.7	206.4	208.3	206.2	213.6	214.6	215.2	223.4	223.1	228.0	229.9	230.0	231.0	231.8	238.0	237.9	237.6	238.1	239.6	238.8	239.2	245.9
	S.D.	33.5	32.5	33.5	35.5	33.9	34.4	38.4	39.4	41.5	39.6	41.2	40.0	39.4	41.1	40.0	42.5	40.8	42.5	40.5	43.9	45.2	42.7
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
500	mean	210.1	215.6	220.6	224.0	224.4	226.1	226.2	229.0	235.5	234.6	240.0	241.0	242.0	245.2	247.5	254.2	252.1	251.5	253.5	252.2	248.7	256.1
	S.D.	45.9	48.8	49.2	51.4	54.1	54.2	55.7	59.2	58.8	54.6	49.3	50.4	50.6	51.4	50.4	49.9	50.7	46.9	43.9	48.5	51.1	48.0
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2500	mean	209.0	213.6	214.1	213.9	214.1	218.0	219.9	226.8	227.2	227.9	232.0	232.7	235.8	238.6	240.8	246.0	247.4	250.8	248.3	247.3	251.3	253.7
	S.D.	32.4	32.5	33.2	35.1	39.4	38.3	35.2	36.1	36.9	38.1	34.8	35.3	36.7	36.2	40.4	40.5	39.2	41.5	38.9	36.3	37.0	36.1
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9

Significantly different from control : *, P<0.05 ; **, P<0.01.

Dose (mg/kg)	Day	Body weight										DEHP										Male (Group 1 and 2)										Unit - B				
		308	315	322	329	336	343	350	357	364	371	378	385	392	399	406	413	420	427	434	441	448	455													
0	mean	266.1	268.8	264.5	266.8	272.5	268.0	262.6	271.1	274.9	268.8	271.4	272.4	271.6	270.4	268.1	277.1	276.1	274.1	270.5	265.0	265.0	263.6													
	S.D.	17.3	18.7	19.5	20.8	20.0	22.0	22.9	21.2	23.0	28.2	22.1	21.9	21.4	26.8	22.8	24.6	23.5	24.3	23.3	14.7	18.0	19.1													
	n	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8													
100	mean	246.4	243.7	247.6	249.2	250.1	256.9	253.3	249.3	244.9	253.8	259.0	263.4	260.4	263.4	263.4	267.1	265.8	267.4	273.6	275.3	273.8	273.1													
	S.D.	41.4	38.4	41.2	40.6	38.5	37.4	38.8	35.8	42.3	40.6	38.9	42.3	46.9	46.5	46.5	46.1	45.8	48.8	48.8	47.8	53.5	54.5													
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9													
500	mean	258.6	260.3	265.4	261.5	269.6	267.6	267.9	268.0	269.8	266.9	262.6	264.5	264.2	264.3	261.6	263.6	261.1	265.1	263.7	259.5	261.9	267.8													
	S.D.	43.4	45.2	45.9	49.7	47.2	45.8	46.9	47.5	45.6	44.8	45.5	42.1	43.2	39.1	40.4	41.5	43.7	45.1	44.7	47.2	44.9	44.7													
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10													
2500	mean	254.7	256.7	252.7	258.9	261.8	262.9	262.4	260.4	266.0	263.1	266.0	264.8	264.3	265.9	268.3	271.3	269.6	269.7	266.1	262.6	268.6	267.1													
	S.D.	36.5	37.9	35.8	38.0	36.6	33.4	38.4	38.1	33.7	37.3	36.2	35.3	34.0	36.0	36.4	36.4	33.8	35.5	34.8	31.1	29.8	30.2													
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9													

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
Table 1
DEHP
Female (Group I)

Dose (mg/kg)	Day	1	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147	
0	mean	143.8	146.3	152.0	149.2	160.3	167.0	169.2	173.3	175.5	181.3	187.2	194.8	201.3	208.3	215.7	214.7	211.2	220.8	225.5	229.0	228.3	227.0	
	S.D.	25.2	30.5	31.9	26.5	32.0	29.0	30.5	27.2	28.5	28.0	32.5	35.7	34.3	32.4	32.2	30.7	33.2	29.2	27.4	27.5	28.0	30.9	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
100	mean	143.8	148.2	155.0	158.7	164.3	168.8	175.2	176.5	182.2	189.0	194.7	200.0	201.0	207.7	212.2	210.7	208.0	209.3	216.3	218.7	229.8	234.3	
	S.D.	16.0	8.1	9.7	8.0	6.6	7.5	9.2	17.6	16.8	19.7	19.3	19.1	19.4	18.9	19.1	23.1	25.3	30.7	29.1	22.3	23.4	22.3	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
500	mean	147.3	144.0	152.7	158.2	157.2	162.7	166.0	168.8	172.2	178.5	178.5	187.3	187.0	188.7	194.2	191.8	194.3	203.8	202.5	210.0	215.8	221.7	
	S.D.	23.0	24.4	24.1	20.2	20.4	23.8	23.7	22.8	24.7	27.6	29.1	29.1	31.1	37.8	35.2	35.7	35.0	37.0	32.5	34.1	36.8	34.2	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
2500	mean	141.8	146.2	152.0	154.4	162.0	166.0	170.0	170.2	173.0	178.2	185.0	188.6	191.2	199.6	202.0	205.8	213.2	217.0	220.8	226.8	230.4	236.6	
	S.D.	16.1	13.2	16.6	14.0	14.2	15.2	16.2	20.5	20.0	20.1	27.9	27.9	31.4	32.3	27.5	29.0	29.6	24.7	23.1	21.8	24.4	26.0	
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
0	mean	230.5	236.5	235.2	236.3	234.0	242.7	242.5	248.8	253.7	258.8	259.5	255.2	259.7	264.2	267.2	267.2	266.8	266.8	274.8	271.8	274.2	271.7	275.5
	S.D.	30.4	29.8	29.3	34.5	30.9	32.3	31.7	32.6	29.5	27.3	30.8	33.0	24.6	31.3	29.6	30.1	31.3	30.8	32.2	29.3	32.6	35.6	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
100	mean	238.5	243.0	246.2	249.7	250.7	252.5	260.0	257.5	259.2	262.3	269.0	269.5	267.2	263.8	268.0	274.2	268.8	266.2	267.7	264.7	253.0	255.7	
	S.D.	24.8	26.3	24.6	25.1	25.1	26.8	26.1	30.8	29.8	27.5	27.3	28.4	36.1	38.8	37.1	32.4	32.4	33.4	29.9	26.4	28.9	25.5	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
500	mean	224.8	227.0	229.5	232.7	239.7	239.8	246.7	251.2	250.0	250.7	252.7	252.8	259.0	262.5	263.0	269.3	272.7	273.5	277.5	278.7	282.2	286.5	
	S.D.	38.0	38.3	41.5	43.6	41.5	41.7	41.9	42.4	39.7	35.4	32.3	32.6	31.6	25.9	22.7	23.3	24.9	24.7	25.3	28.5	27.0	27.9	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
2500	mean	235.2	240.4	243.0	248.2	254.0	256.2	257.6	259.4	260.8	264.6	267.4	266.4	272.0	270.2	273.4	275.2	276.8	276.8	278.8	278.2	275.6	279.0	
	S.D.	23.8	17.9	13.8	17.4	16.4	19.2	16.4	16.1	17.5	17.5	15.8	18.8	16.4	16.5	18.3	15.9	14.2	20.6	18.4	18.1	19.4	14.4	
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
Table 1
DEHP
Female (Group 1)

Dose (mg/kg)	Day	308	315	322	329	336	343	350	357	364	371	378	385	392	399	406	413	420	427	434	441	448	455
0	mean	276.5	276.7	277.8	280.3	274.7	272.7	276.0	271.2	276.5	282.0	276.8	280.3	280.3	278.5	276.0	281.2	275.7	275.2	279.5	277.0	284.7	281.0
	S.D.	38.7	42.3	39.5	36.4	38.7	39.3	38.5	44.3	41.4	40.2	40.8	44.7	38.8	43.4	51.9	40.5	40.8	41.5	39.8	38.7	43.5	42.2
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
100	mean	259.0	260.2	259.2	254.5	263.3	257.8	257.3	253.2	256.2	265.0	260.3	263.3	261.8	258.3	257.5	260.0	265.0	265.2	266.3	266.7	275.5	269.7
	S.D.	22.7	16.5	16.0	13.3	14.8	16.7	17.4	16.6	11.2	12.7	14.6	15.5	18.0	12.1	19.9	21.6	19.6	17.7	20.4	20.9	25.3	30.1
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
500	mean	291.2	292.3	291.2	295.5	298.3	295.5	295.7	296.8	297.5	299.3	296.2	299.3	306.2	308.7	309.3	316.8	315.8	317.5	318.7	317.2	319.7	316.0
	S.D.	27.1	23.2	25.1	26.2	26.8	27.8	26.1	24.1	23.5	26.8	31.3	30.4	27.6	26.5	25.5	28.4	28.7	31.7	32.8	32.9	33.9	36.5
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
2500	mean	271.4	285.2	281.8	286.0	286.6	286.8	280.8	286.6	288.8	291.6	292.0	295.6	295.4	295.8	286.8	287.8	288.4	289.8	295.0	297.4	299.2	296.8
	S.D.	31.2	11.1	12.5	14.1	15.2	12.9	22.5	19.3	19.0	16.0	19.4	17.3	16.1	17.4	28.4	31.6	26.2	25.5	22.7	19.4	19.5	20.2
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Significantly different from control : * , P<0.05 ; ** , P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
Table 1
Male (Group 5)

Dose (mg/kg)	Body Weight																						
	Day 1	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147	
100	mean	136.7	149.3	144.7	145.0	159.3	164.0	172.0	176.7	183.3	187.7	198.3	204.3	211.7	214.3	216.0	222.0	221.3	223.0	221.7	231.7	239.3	240.0
	S.D.	21.6	19.9	26.5	24.3	19.7	19.3	18.5	20.2	21.6	24.0	18.1	18.3	19.1	28.3	29.5	18.1	7.6	8.0	5.9	9.1	5.5	8.9
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2500	mean	144.0	144.7	150.0	148.0	158.7	156.0	165.3	171.7	168.0	174.7	184.7	183.0	194.7	202.7	208.0	212.7	211.7	205.3	206.7	219.3	211.3	223.7
	S.D.	19.7	21.6	12.3	22.9	21.5	25.0	27.7	27.6	29.7	27.2	28.0	35.0	29.8	33.4	31.2	35.4	45.2	43.4	55.6	53.4	59.7	59.2
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
100	mean	239.3	252.0	259.3	264.3	266.3	259.0	265.7	273.3	280.3	280.7	280.0	280.0	283.0	280.7	288.7	277.3	283.7	282.0	279.7	119.7	297.3	289.3
	S.D.	14.4	9.2	11.0	15.6	13.2	23.6	13.7	13.1	9.0	11.0	11.1	13.5	17.3	28.0	22.0	27.0	24.4	27.5	36.9	159.7	10.6	19.9
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2500	mean	224.3	232.3	224.0	230.7	228.3	222.7	231.3	231.0	230.7	228.7	234.3	236.3	239.3	243.0	251.7	256.3	260.3	257.3	262.0	267.3	265.3	261.7
	S.D.	57.8	60.3	66.7	65.1	68.1	74.9	65.8	61.9	62.7	60.9	63.8	63.8	66.8	66.3	62.7	58.0	57.1	53.5	54.8	53.4	53.4	58.1
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
100	mean	288.0	297.0	304.3	300.0	296.3	303.3	304.0	306.0	289.3	306.3	298.0	300.3	298.7	287.7	277.7	286.7	281.3	291.7	296.3	303.7	309.0	307.3
	S.D.	19.3	6.2	4.2	7.8	11.6	10.1	7.8	11.3	8.1	7.0	18.2	17.2	9.5	15.3	15.0	8.1	21.7	21.0	17.0	19.6	25.2	23.5
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2500	mean	258.3	269.3	264.0	262.7	269.7	273.3	273.0	270.0	271.0	277.0	278.7	268.3	275.0	279.0	262.3	280.7	284.0	276.7	281.7	284.0	279.0	279.0
	S.D.	52.2	43.5	51.2	35.8	35.4	33.9	34.2	24.6	27.0	33.1	22.9	45.7	27.5	36.1	48.1	24.7	29.7	17.0	22.3	21.9	12.1	21.8
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
Female (Group 5)
DEHP

Dose (mg/kg)	Day	1	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140	147
100	mean	142.0	136.7	149.0	152.7	160.3	164.7	168.0	168.0	174.0	173.0	181.3	171.0	178.3	166.0	165.7	169.7	165.7	171.7	169.7	170.0	170.3	173.3
	S.D.	29.5	29.0	34.8	42.6	43.4	43.4	43.6	46.8	47.7	53.1	52.0	44.7	50.2	38.7	42.0	42.9	40.3	42.9	40.2	44.9	40.4	36.8
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2500	mean	149.3	147.0	154.3	161.3	165.0	170.3	175.3	180.3	187.3	190.0	198.0	200.0	199.0	189.3	201.7	209.0	199.7	171.7	205.7	212.3	218.0	221.7
	S.D.	18.0	25.9	24.0	18.6	10.8	14.7	14.0	17.0	12.3	8.5	10.5	7.5	13.9	13.4	11.2	10.0	10.1	42.9	6.8	6.7	13.1	3.1
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Dose (mg/kg)	Day	154	161	168	175	182	189	196	203	210	217	224	231	238	245	252	259	266	273	280	287	294	301
100	mean	174.7	170.7	177.0	176.3	173.3	184.3	184.3	185.7	195.0	195.7	201.3	206.7	212.3	214.3	221.0	220.0	221.7	226.3	235.7	242.0	242.7	242.0
	S.D.	36.2	35.6	34.6	34.8	40.5	37.0	35.5	30.1	38.7	27.5	30.3	27.5	28.1	24.7	25.2	28.0	30.9	24.1	34.8	39.2	45.0	43.3
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2500	mean	213.7	231.0	231.7	228.0	219.0	227.3	227.3	228.7	229.0	234.7	243.0	243.7	247.7	250.7	254.0	254.3	258.3	264.7	265.7	270.0	264.7	266.7
	S.D.	4.6	5.3	5.8	8.7	9.2	11.7	30.7	20.0	12.0	18.1	5.2	5.5	12.7	12.9	17.1	19.0	18.0	22.0	28.4	27.6	35.4	33.0
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Dose (mg/kg)	Day	308	315	322	329	336	343	350	357	364	371	378	385	392	399	406	413	420	427	434	441	448	455
100	mean	243.3	246.3	252.0	249.0	257.3	260.3	259.7	264.7	268.7	270.0	270.0	271.0	276.0	275.3	278.7	281.7	283.3	283.7	283.0	288.3	289.7	283.3
	S.D.	48.2	52.4	52.3	57.4	55.2	56.2	61.1	61.0	66.2	65.5	65.5	67.2	63.3	62.4	67.3	66.3	64.3	67.2	67.3	68.7	71.0	72.9
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2500	mean	273.7	275.0	282.0	280.3	287.7	282.3	286.7	289.3	281.3	284.3	285.3	290.7	288.0	285.3	286.3	292.3	286.7	290.0	289.7	290.3	293.7	289.3
	S.D.	28.2	26.7	25.9	29.3	22.2	21.9	20.5	29.0	18.9	32.3	28.4	28.0	30.5	29.8	31.8	26.5	32.6	32.0	27.6	34.1	29.2	30.9
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets (Group 1 and 2)

Dose (mg/kg)	Hematology									
	DEHP					Male				
	Erythrocyte count	Hemoglobin conc.	Hematocrit	MCV	MCH	MCHC	Platelet Count	Reticulocyte Ratio		
	×10E6/μL	g/dL	fL	pg	g/dL	×10E3/μL	%	%	%	%
	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65
0	mean	6.52	14.4	49.8	76.6	22.1	647	5.3		
	S.D.	0.63	1.1	3.4	4.1	1.2	78	2.9		
	n	8	8	8	8	8	8	8		
100	mean	6.56	14.5	51.0	77.9	22.0	689	6.2		
	S.D.	0.88	2.0	6.5	3.9	0.4	254	3.2		
	n	9	9	9	9	9	9	9		
500	mean	6.15	13.9	48.4	79.2	22.8	688	6.7		
	S.D.	0.84	1.5	4.1	6.0	1.3	184	3.6		
	n	10	10	10	10	10	10	10		
2500	mean	6.55	14.6	50.2	77.7	22.5	632	6.5		
	S.D.	1.09	1.6	4.6	7.5	1.5	127	4.2		
	n	9	9	9	9	9	9	9		

Dose (mg/kg)	Leucocyte count											
	Lymphocyte		Neutrophilic Segmented		Neutrophilic Band		Eosinophil		Basophil		Monocyte	
	%	%	%	%	%	%	%	%	%	%	%	
	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	Week 65	
0	mean	38.1	52.2	3.3	1.5	0.5	4.5					
	S.D.	11.8	10.2	2.8	2.7	0.9	3.4					
	n	8	8	8	8	8	8	8				
100	mean	59.2*	34.3	2.5	0.3	0.4	3.3					
	S.D.	17.9	17.3	1.9	0.5	0.7	3.2					
	n	9	9	9	9	9	9	9				
500	mean	44.3	47.1	4.8	0.5	0.6	2.6					
	S.D.	14.3	13.1	4.8	1.1	0.8	1.4					
	n	10	10	10	10	10	10	10				
2500	mean	47.1	43.8	3.2	1.7	0.8	3.5					
	S.D.	12.7	12.5	1.9	1.8	0.7	2.6					
	n	9	9	9	9	9	9	9				

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets (Group 1)
Female
DEHP

Dose (mg/kg)	Erythrocyte count		Hemoglobin conc.		Hematocrit		MCV		MCH		MCHC		Platelet Count		Reticulocyte Ratio	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
0	6.33	0.51	14.6	1.2	49.3	4.0	77.9	2.4	23.0	0.3	29.6	1.1	671	118	5.6	1.5
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
100	6.28	0.30	14.5	0.7	49.7	2.4	79.2	2.9	23.1	0.8	29.2	0.5	763	127	5.5	2.1
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
500	7.24	0.80	16.2	1.2	53.6	3.9	74.5	4.3	22.4	1.0	30.1	0.6	576	99	4.1	1.6
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
2500	6.30	0.96	14.6	2.0	49.1	5.8	78.3	3.0	23.2	0.5	29.6	0.9	671	163	5.4	3.2
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Dose (mg/kg)	Leucocyte count		Lymphocyte		Neutrophilic Segmented		Neutrophilic Band		Eosinophil		Basophil		Monocyte	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
0	6.42	1.73	42.6	11.5	49.4	13.2	1.5	0.8	2.3	1.5	0.5	0.8	3.7	2.0
	n	6	6	6	6	6	6	6	6	6	6	6	6	6
100	6.86	1.04	39.8	14.5	47.8	19.3	5.7	5.0	1.3	1.5	0.5	0.6	4.9	4.1
	n	6	6	6	6	6	6	6	6	6	6	6	6	6
500	5.89	2.19	56.1	16.6	37.0	14.6	1.7	2.0	1.9	1.5	0.5	0.6	2.9	1.4
	n	6	6	6	6	6	6	6	6	6	6	6	6	6
2500	7.35	2.01	57.4	7.6	35.0	5.6	2.8	2.8	1.2	0.8	0.4	0.5	3.2	1.7
	n	5	5	5	5	5	5	5	5	5	5	5	5	5

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets (Group 1 and 2)
 Blood Chemistry Male

Dose (mg/kg)	ASAT(GOT)		ALAT(GPT)		LDH		γGt		ALP		Total Bilirubin		Urea Nitrogen		Creatinine		Glucose		Total Cholesterol		Free Cholesterol		Phospholipid		Triglyceride	
	U/L	Week 65	U/L	Week 65	U/L	Week 65	U/L	Week 65	U/L	Week 65	mg/dL	Week 65	mg/dL	Week 65	mg/dL	Week 65	mg/dL	Week 65	mg/dL	Week 65	mg/dL	Week 65	mg/dL	Week 65	mg/dL	Week 65
0	mean	127	2	177	7	751	0.1	25.8	0.3	93	180	46	197	57												
	S.D.	32	2	43	2	515	0.1	5.9	0.1	16	21	5	40	21												
	n	8	8	8	8	8	8	8	8	8	8	8	8	8												
100	mean	115	2	145	8	706	0.1	21.2	0.2	101	184	44	207	43												
	S.D.	27	1	23	3	244	0.0	5.9	0.1	16	35	7	42	12												
	n	9	9	9	9	9	9	9	9	9	9	9	9	9												
500	mean	112	2	139	10	715	0.1	25.7	0.2	100	154	41	171	49												
	S.D.	33	2	23	5	282	0.1	5.4	0.0	33	20	6	25	29												
	n	10	10	10	10	10	10	10	10	10	10	10	10	10												
2500	mean	127	2	156	10	599	0.2	24.3	0.2	94	156	40	176	41												
	S.D.	43	1	51	5	242	0.1	4.4	0.0	20	32	7	35	8												
	n	9	9	9	9	9	9	9	9	9	9	9	9	9												

Dose (mg/kg)	Total Protein		Albumin		A/G Ratio		Calcium		Inorganic Phosphorus		Na		K		Cl	
	g/dL	Week 65	g/dL	Week 65	Week 65	Week 65	mg/dL	Week 65	mg/dL	Week 65	mmol/L	Week 65	mmol/L	Week 65	mmol/L	Week 65
0	mean	6.2	2.4	0.64	9.2	5.3	154	5.1	108							
	S.D.	0.9	0.4	0.08	0.5	1.2	3	0.4	3							
	n	8	8	8	8	8	8	8	8							
100	mean	6.7	2.8	0.71	9.7	4.8	155	5.2	108							
	S.D.	1.1	0.7	0.11	0.6	1.0	5	0.6	3							
	n	9	9	9	9	9	9	9	9							
500	mean	7.2	2.9	0.67	9.6	4.9	153	5.1	107							
	S.D.	0.8	0.5	0.13	0.5	0.5	4	0.6	3							
	n	10	10	10	10	10	10	10	10							
2500	mean	7.1	3.0	0.73	9.7	4.8	156	5.2	110							
	S.D.	0.8	0.3	0.07	0.6	0.5	2	0.4	1							
	n	9	9	9	9	9	9	9	9							

Significantly different from control : * , P<0.05 ; ** , P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets (Group 1)
Female

Dose (mg/kg)	Blood Chemistry												
	ASAT(GOT) U/L Week 65	ALAT(GPT) U/L Week 65	LDH U/L Week 65	γ Gt U/L Week 65	ALP U/L Week 65	Total Bilirubin mg/dL Week 65	Urea Nitrogen mg/dL Week 65	Creatinine mg/dL Week 65	Glucose mg/dL Week 65	Total Cholesterol mg/dL Week 65	Free Cholesterol mg/dL Week 65	Phospholipid mg/dL Week 65	Triglyceride mg/dL Week 65
0	mean	2	126	10	548	0.1	17.3	0.2	102	159	40	188	52
	S.D.	1	16	5	129	0.0	8.5	0.0	15	16	3	28	11
	n	6	6	6	6	6	6	6	6	6	6	6	6
100	mean	1	135	6	648	0.1	23.5	0.2	110	146	38	172	47
	S.D.	1	25	3	273	0.1	4.6	0.0	36	22	5	20	21
	n	6	6	6	6	6	6	6	6	6	6	6	6
500	mean	8	204	14	564	0.2	21.2	0.3	95	151	35	183	41
	S.D.	11	94	7	127	0.1	5.7	0.1	17	29	5	30	9
	n	6	6	6	6	6	6	6	6	6	6	6	6
2500	mean	2	140	11	462	0.2	19.8	0.2	100	163	39	189	41
	S.D.	1	15	9	96	0.1	2.1	0.0	8	28	5	23	7
	n	5	5	5	5	5	5	5	5	5	5	5	5

Dose (mg/kg)	Blood Chemistry									
	Total Protein g/dL Week 65	Albumin g/dL Week 65	A/G Ratio Week 65	Calcium mg/dL Week 65	Inorganic Phosphorus mg/dL Week 65	Na mmol/L Week 65	K mmol/L Week 65	Cl mmol/L Week 65		
0	mean	6.6	2.8	0.72	9.5	4.3	4.8	109		
	S.D.	0.5	0.5	0.13	0.4	0.8	0.5	2		
	n	6	6	6	6	6	6	6		
100	mean	6.2	2.4	0.63	9.0	5.5	5.0	108		
	S.D.	1.1	0.5	0.12	0.7	1.3	0.4	3		
	n	6	6	6	6	6	6	6		
500	mean	7.6	3.4	0.81	10.2	4.3	5.0	111		
	S.D.	1.1	0.7	0.11	0.9	1.3	0.6	4		
	n	6	6	6	6	6	6	6		
2500	mean	7.0	3.1	0.77	9.7	4.1	5.0	111		
	S.D.	1.5	0.8	0.10	1.1	0.7	0.5	1		
	n	5	5	5	5	5	5	5		

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Hormonal Analysis
 Male

Dose (mg/kg)	Week	Testosterone (ng/mL)					Estradiol (pg/mL)						
		Pre	13	26	39	52	65	Pre	13	26	39	52	65
0	mean	0.10	2.47	7.61	0.65	6.89	3.93	<10.0	13.4	17.7	10.4	23.8	16.2
	S.D.	0.03	4.66	9.01	1.23	8.42	8.97		8.3	9.4	0.9	14.7	13.1
	n	6	6	6	6	6	6	6	6	6	6	6	6
100	mean	0.08	0.12	3.19	1.37	3.29	8.12	10.2	<10.0	16.7	13.1	25.3	30.1
	S.D.	0.03	0.15	7.60	3.14	5.73	10.09	0.6	6	16.4	7.6	32.3	31.2
	n	6	6	6	6	6	6	6	6	6	6	6	6
500	mean	0.35	0.27	4.85	0.80	0.92	6.56	<10.0	<10.0	18.3	12.2	17.3	20.3
	S.D.	0.54	0.35	9.51	1.23	1.13	6.61			19.8	5.9	9.6	16.1
	n	7	7	7	7	7	7	7	7	7	7	7	7
2500	mean	0.69	0.49	0.54	1.70	2.17	3.29	10.2	<10.0	11.2	<10.0	10.1	16.8
	S.D.	1.36	1.08	1.14	1.78	4.83	5.33	0.5	6	2.4		0.3	16.7
	n	6	6	6	6	6	6	6	6	6	6	6	6

The value of the quantitation limit was used for calculating mean and S.D.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Hormonal Analysis
 DEHP
 Male

Dose (mg/kg)	Week	T ₃ (ng/dL)					T ₃ (μg/dL)						
		Pre	13	26	39	52	65	Pre	13	26	39	52	65
0	mean	376.0	360.7	303.0	200.0	254.1	129.9	16.29	14.96	10.42	11.81	13.79	12.56
	S.D.	187.5	98.9	104.9	51.7	137.5	33.5	5.57	4.20	3.71	2.56	2.91	4.29
	n	6	6	6	6	6	6	6	6	6	6	6	6
100	mean	328.9	352.4	326.6	137.0	203.8	223.4	14.33	12.46	11.49	12.90	13.70	12.95
	S.D.	111.2	187.7	172.4	48.9	133.2	119.2	3.43	1.89	2.03	1.91	1.59	2.03
	n	6	6	4	6	6	6	6	6	5	6	6	6
500	mean	298.8	253.5	235.7	273.1	235.2	237.8	17.84	14.57	13.12	13.41	12.65	11.80
	S.D.	137.0	155.9	124.3	179.7	113.6	72.7	5.78	3.33	4.22	2.60	1.29	1.06
	n	7	7	6	7	7	7	7	7	7	7	7	7
2500	mean	428.4	275.1	198.4	236.6	151.5	129.9	16.04	16.56	11.56	14.73	12.58	11.04
	S.D.	100.3	94.7	115.0	152.7	36.9	37.9	2.09	2.55	1.69	1.23	1.28	1.51
	n	6	6	6	6	6	6	6	6	5	6	6	6

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 4 Hormonal Analysis
 DEHP Female

Dose (mg/kg)	Week	Testosterone(ng/mL)					Estradiol(pg/mL)						
		Pre	13	26	39	52	65	Pre	13	26	39	52	65
0	mean	0.05	<0.05	0.09	0.08	0.06	0.11	<10.0	11.1	17.5	85.4	28.7	124.0
	S.D.	0.00		0.03	0.06	0.02	0.05		2.7	11.8	166.6	29.2	251.3
	n	6	6	6	6	6	6	6	6	6	6	6	6
100	mean	0.12	0.05	0.06	0.08	0.11	0.17	<10.0	<10.0	20.5	16.3	25.9	236.7
	S.D.	0.14	0.01	0.02	0.04	0.08	0.16		6	14.9	11.2	17.8	371.5
	n	6	6	6	6	6	6	6	6	6	6	6	6
500	mean	0.14	0.06	0.08	0.15	0.27	0.20	<10.0	11.9	21.9	49.5	484.3	891.9
	S.D.	0.17	0.02	0.02	0.12	0.24	0.09		4.0	17.1	46.3	648.7	292.1
	n	6	6	6	6	6	6	6	6	6	6	6	6
2500	mean	0.14	<0.05	0.06	0.08	0.10	0.13	<10.0	11.4	26.1	52.3	268.8	575.8
	S.D.	0.19		0.01	0.04	0.05	0.07		3.0	31.8	38.8	448.8	581.1
	n	5	5	5	5	5	5	5	5	5	5	5	5

The value of the quantitation limit was used for calculating mean and S.D.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 4
 Hormonal Analysis
 DEHP
 Female

Study No. B0000496

Dose (mg/kg)	Week	T ₃ (ng/dL)					T ₄ (μg/dL)						
		Pre	13	26	39	52	65	Pre	13	26	39	52	65
0	mean	360.5	404.6	174.6	239.8	179.4	241.6	14.36	13.12	11.22	12.50	13.19	12.62
	S.D.	133.5	99.5	96.5	104.1	31.6	104.2	2.77	5.02	1.83	2.66	2.98	3.12
	n	6	6	4	6	6	6	6	6	6	5	6	6
100	mean	398.4	385.9	206.1	167.5	168.7	139.6	16.72	15.02	12.24	13.13	13.60	12.48
	S.D.	130.8	96.4	45.4	76.8	84.6	81.6	3.60	1.89	2.64	1.84	1.14	3.33
	n	6	6	4	6	6	6	6	6	6	6	6	6
500	mean	346.8	325.2	298.4	253.8	299.1	281.7	16.74	13.94	12.17	11.78	12.49	12.52
	S.D.	100.0	147.8	124.2	161.4	97.7	81.4	5.54	3.97	0.93	1.87	2.51	2.16
	n	6	5	4	6	6	6	6	6	5	6	6	6
2500	mean	302.2	293.7	267.9	203.9	214.1	188.7	13.59	13.13	13.51	11.77	11.65	10.90
	S.D.	142.9	140.6	79.4	78.8	91.5	71.3	1.45	4.66	2.29	2.78	1.70	3.75
	n	5	4	4	5	5	5	5	4	5	5	5	5

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common marmosets
 Table 5 Organ Weight

Study No. B000496

Male

DEHP

Dose (mg/kg)	Final Body Weight		Pituitary		Thyroid		Thyroid Right		Thyroids		Liver		Spleen		Pancreas		Kidney Left		Kidney Right		Kidneys	
	Week 66	g	mg	mg	mg	mg	mg	mg	mg	mg	g	g	g	g	g	g	g	g	g	g	g	g
0	mean	251	11.8	33.0	29.9	62.9	9.90	0.23	0.66	0.76	0.76	1.52	0.76	0.76	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
	S.D.	23	1.9	17.8	8.3	25.9	1.99	0.06	0.15	0.16	0.16	0.31	0.16	0.16	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
100	mean	267	11.1	32.1	31.4	63.5	10.48	0.23	0.69	0.78	0.78	1.59	0.78	0.78	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
	S.D.	34	1.8	6.6	10.1	14.8	2.32	0.05	0.15	0.13	0.13	0.26	0.13	0.13	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
500	mean	262	10.1	34.4	32.0	66.4	9.93	0.23	0.79	0.92	0.92	1.85	0.92	0.92	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
	S.D.	53	1.2	10.0	7.0	15.9	2.32	0.11	0.18	0.23	0.23	0.44	0.23	0.23	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
2500	mean	249	11.7	31.2	31.8	63	9.09	0.22	0.60	0.84	0.84	1.75	0.84	0.84	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
	S.D.	34	2.0	6.2	5.4	11.4	1.13	0.08	0.17	0.09	0.09	0.21	0.09	0.09	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

Significantly different from control : *, P<0.05; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common marmosets
 Table 5 Organ Weight
 DEHP Female

Dose (mg/kg)	Final Body Weight		Pituitary		Thyroid Left		Thyroid Right		Thyroids		Liver		Spleen		Pancreas		Kidney Left		Kidney Right		Kidneys	
	Week 66	g	mg	mg	mg	mg	mg	mg	mg	mg	g	g	g	g	g	g	g	g	g	g	g	g
0	mean	276	11.3	35.6	33.6	69.2	12.93	0.25	0.88	0.96	1.84	0.88	0.96	1.84	0.88	0.96	1.84	0.88	0.96	1.84	0.88	0.96
	S.D.	34	2.4	10.0	9.9	19.4	5.53	0.08	0.22	0.15	0.36	0.22	0.15	0.36	0.22	0.15	0.36	0.22	0.15	0.36	0.22	0.15
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
100	mean	265	10.7	30.6	29.8	60.4	10.19	0.21	0.83	0.87	1.75	0.83	0.87	1.75	0.83	0.87	1.75	0.83	0.87	1.75	0.83	0.87
	S.D.	21	1.0	9.6	6.7	15.2	2.50	0.05	0.16	0.10	0.21	0.16	0.05	0.10	0.16	0.10	0.21	0.16	0.10	0.21	0.16	0.10
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
500	mean	307	11.2	34.9	34.9	69.8	12.17	0.34	0.80	0.96	1.92	0.80	0.96	1.92	0.80	0.96	1.92	0.80	0.96	1.92	0.80	0.96
	S.D.	30	2.2	11.2	8.0	18.7	1.70	0.17	0.13	0.11	0.22	0.13	0.11	0.22	0.13	0.11	0.22	0.13	0.11	0.22	0.13	0.11
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
2500	mean	293	11.4	36.3	40.2	76.5	12.07	0.24	1.00	0.93	1.89	1.00	0.93	1.89	1.00	0.93	1.89	1.00	0.93	1.89	1.00	0.93
	S.D.	18	1.1	9.5	9.9	18.7	1.39	0.09	0.18	0.08	0.16	0.18	0.09	0.08	0.16	0.09	0.08	0.16	0.09	0.08	0.16	0.09
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
0	mean	68.3	66.8	135.1	60.9	75.6	136.5	0.16	136.5	75.6	0.16	136.5	0.16	136.5	75.6	0.16	136.5	75.6	0.16	136.5	75.6	0.16
	S.D.	13.1	11.4	24.3	15.2	43.3	52.8	0.04	52.8	43.3	0.04	52.8	0.04	52.8	43.3	0.04	52.8	43.3	0.04	52.8	43.3	0.04
100	mean	67.6	68	135.6	67.3	69.8	137.1	0.17	137.1	69.8	0.17	137.1	0.17	137.1	69.8	0.17	137.1	69.8	0.17	137.1	69.8	0.17
	S.D.	13.3	8.0	20.7	26.1	46.2	64.0	0.07	64.0	46.2	0.07	64.0	0.07	64.0	46.2	0.07	64.0	46.2	0.07	64.0	46.2	0.07
500	mean	73.8	71.4	145.2	105.7*	139.9*	245.6*	0.30*	245.6*	139.9*	0.30*	245.6*	0.30*	245.6*	139.9*	0.30*	245.6*	139.9*	0.30*	245.6*	139.9*	0.30*
	S.D.	7.8	5.5	12.2	24.3	41.0	50.5	0.09	50.5	41.0	0.09	50.5	0.09	50.5	41.0	0.09	50.5	41.0	0.09	50.5	41.0	0.09
2500	mean	68.8	61.1	129.8	106.3	124.2	230.5*	0.27	230.5*	124.2	0.27	230.5*	0.27	230.5*	124.2	0.27	230.5*	124.2	0.27	230.5*	124.2	0.27
	S.D.	9.1	3.2	11.8	47.2	28.0	64.9	0.11	64.9	28.0	0.11	64.9	0.11	64.9	28.0	0.11	64.9	28.0	0.11	64.9	28.0	0.11
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Significantly different from control : *, P<0.05; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common marmosets
 Table 6
 Relative Organ Weight
 DEHP
 Male

Dose (mg/kg)	Final Body Weight		Pituitary		Thyroid Left		Thyroid Right		Thyroids		Liver		Spleen		Pancreas		Kidney Left		Kidney Right		Kidneys	
	Week 66	g	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66	%
0	mean	251	4.7	13.3	12.0	25.4	3.92	0.09	0.27	0.31	0.30	0.61										
	S.D.	23	0.6	7.7	3.8	11.3	0.55	0.02	0.06	0.06	0.05	0.11										
	n	5	5	5	5	5	5	5	5	5	5	5										
100	mean	267	4.2	12.4	11.8	24.2	3.95	0.09	0.26	0.29	0.31	0.60										
	S.D.	34	0.9	3.9	3.6	6.7	0.94	0.02	0.07	0.04	0.07	0.10										
	n	6	6	6	6	6	6	6	6	6	6	6										
500	mean	262	3.9	13.3	12.4	25.7	3.79	0.08	0.31	0.35	0.35	0.71										
	S.D.	53	0.6	3.9	2.7	6.1	0.38	0.03	0.07	0.04	0.03	0.06										
	n	7	7	7	7	7	7	7	7	7	7	7										
2500	mean	249	4.7	12.5	12.8	25.2	3.67	0.09	0.25	0.34	0.37	0.71										
	S.D.	34	0.3	1.5	1.1	2.3	0.26	0.04	0.10	0.02	0.03	0.05										
	n	6	6	6	6	6	6	6	6	6	6	6										

Dose (mg/kg)	Adrenal Left		Adrenal Right		Adrenals		Testis Left		Testis Right		Testes		Prostate		Seminal Vesicles		Epididymis Left		Epididymis Right		Epididymides	
	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66	%
0	mean	23.1	21.4	44.5	0.17	0.17	0.33	0.04	0.17	0.17	0.33	0.04	0.02	0.04	0.02	0.03	0.03	0.03	0.03	0.06	0.06	
	S.D.	6.1	5.4	11.4	0.04	0.04	0.07	0.02	0.04	0.04	0.07	0.02	0.01	0.02	0.01	0.01	0.00	0.00	0.01	0.01		
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
100	mean	21.4	20.8	42.1	0.13	0.13	0.27	0.03	0.13	0.13	0.27	0.03	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.06	0.06	
	S.D.	5.3	4.3	9.7	0.04	0.04	0.09	0.01	0.05	0.05	0.09	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
500	mean	24.1	22.0	46.0	0.13	0.14	0.27	0.04	0.14	0.14	0.27	0.04	0.02	0.04	0.02	0.02	0.03	0.03	0.03	0.06	0.06	
	S.D.	4.6	3.5	7.6	0.03	0.03	0.07	0.03	0.04	0.04	0.07	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
2500	mean	24.2	22.9	47.1	0.14	0.15	0.29	0.03	0.15	0.15	0.29	0.03	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.06	0.06	
	S.D.	6.4	4.2	10.6	0.08	0.08	0.16	0.01	0.08	0.08	0.16	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.02	0.02	
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common marmosets
 DEHP
 Female
 Table 6
 Relative Organ Weight

Dose (mg/kg)	Final Body Weight		Pituitary		Thyroid		Thyroids		Liver		Spleen		Pancreas		Kidney Left		Kidney Right		Kidneys	
	mean	S.D.	g	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66	%	Week 66
0	mean	276	4.1	13.1	12.5	25.6	4.76	0.09	0.32	0.31	0.35	0.66								
	S.D.	34	0.8	4.3	4.7	8.9	2.22	0.02	0.08	0.06	0.03	0.09								
	n	6	6	6	6	6	6	6	6	6	6	6	6							
100	mean	265	4.1	11.5	11.2	22.7	3.92	0.08	0.32	0.33	0.33	0.66								
	S.D.	21	0.5	3.1	2.0	4.7	1.29	0.02	0.08	0.05	0.04	0.08								
	n	6	6	6	6	6	6	6	6	6	6	6	6							
500	mean	307	3.7	11.2	11.3	22.6	3.98	0.11	0.26	0.31	0.32	0.63								
	S.D.	30	0.7	2.9	2.3	4.9	0.51	0.07	0.03	0.05	0.04	0.09								
	n	6	6	6	6	6	6	6	6	6	6	6	6							
2500	mean	293	3.9	12.3	13.6	25.9	4.13	0.08	0.34	0.32	0.32	0.64								
	S.D.	18	0.4	2.4	2.7	4.7	0.52	0.02	0.07	0.03	0.02	0.05								
	n	5	5	5	5	5	5	5	5	5	5	5	5							

Dose (mg/kg)	Adrenals		Adrenal Right		Adrenal Left		Ovaries		Uterus	
	mean	S.D.	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	$\times 10^{-3}\%$	Week 66	%	Week 66
0	mean	25.3	50.0	24.7	21.9	48.3	0.06			
	S.D.	6.9	13.4	6.6	4.0	13.8	0.01			
	n	6	6	6	6	6	6			
100	mean	25.7	51.5	25.8	25.2	51.0	0.06			
	S.D.	5.5	8.6	3.3	8.7	21.2	0.02			
	n	6	6	6	6	6	6			
500	mean	24.2	47.7	23.4	35.0	80.7*	0.10*			
	S.D.	3.8	6.2	2.6	10.1	18.1	0.03			
	n	6	6	6	6	6	6			
2500	mean	23.5	44.4	20.9	35.8	78.2*	0.09			
	S.D.	2.6	3.6	1.5	13.8	18.5	0.04			
	n	5	5	5	5	5	5			

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common marmosets
 Table 7 Sperm Counting (Testis) DEHP Male

Dose (mg/kg)	Sperm Count ($\times 10^6/g$)		
0	mean	4.0	
	S.D.	1.5	
	n	5	
100	mean	4.4	
	S.D.	1.2	
	n	5	
500	mean	4.0	
	S.D.	1.8	
	n	6	
2500	mean	4.5	
	S.D.	0.8	
	n	5	

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 8 Testicular Enzyme Activities and Components DEHP Male

Dose (mg/kg)	SDHI		γ GT		Total glutathione content		Glutathione S-transferase activity		Glutathione peroxidase activity		Zn	
	U/L	U/L	U/L	U/L	μ mol/g testis	μ mol / mg pro / min	μ mol / g testis / min	nmol / mg pro / min	μ mol / g testis / min	μ g / g	μ g / g	Week 66
0	mean	3074.4	218.4	218.4	2.00	0.40	19.6	48.4	2.4	13.9		
	S.D.	92.3	65.7	65.7	0.11	0.03	2.5	1.8	0.3	1.8		
	n	5	5	5	5	5	5	5	5	5		
100	mean	2658.6	247.8	247.8	1.54	0.38	19.4	53.4	2.7	11.7 *		
	S.D.	704.6	80.5	80.5	0.45	0.04	2.9	3.8	0.2	1.2		
	n	5	5	5	5	5	5	5	5	5		
500	mean	2439.5	276.5	276.5	1.61	0.32 *	16.2	51.0	2.6	11.1 **		
	S.D.	556.3	67.0	67.0	0.39	0.02	1.8	4.4	0.4	0.9		
	n	6	6	6	6	6	6	6	6	7		
2500	mean	2595.6	247.8	247.8	1.52	0.34	16.4	53.0	2.5	13.0		
	S.D.	441.6	110.5	110.5	0.29	0.04	0.9	8.6	0.4	1.7		
	n	5	5	5	5	5	5	5	5	6		

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate (DEHP) in Juvenile Common Marmosets
 Table 9 Hepatic Enzyme Activities and Components DEHP Male

Dose (mg/kg)	FAOS		CAT		CPT		Cytochrome P450 content		Testosterone 6 β -hydroxylase activity		Lauric acid ω -1-hydroxylase activity		Lauric acid ω -hydroxylase activity	
	U/mg-pro Week 66	pmol/mg pro Week 66	nmol/g liver Week 66	nmol/mg pro/min Week 66	nmol/g liver/min Week 66	nmol/mg pro/min Week 66	nmol/g liver/min Week 66	nmol/mg pro/min Week 66	nmol/g liver/min Week 66	nmol/mg pro/min Week 66				
0	mean	0.41	28.9	5.50	208.6	4.1	1.78	34.9	0.449	8.8	0.360	7.0		
	S.D.	0.17	13.1	1.73	89.5	2.5	0.55	16.9	0.114	3.9	0.082	2.8		
	n	5	5	5	5	5	5	5	5	5	5	5		
100	mean	0.39	24.5	5.59	327.2	7.9	2.45	59.5	0.518	12.5	0.420	10.0		
	S.D.	0.15	7.7	2.19	51.8	2.6	0.61	23.5	0.102	3.9	0.050	2.9		
	n	6	6	6	6	6	6	6	6	6	6	6		
500	mean	0.34	21.7	4.38	292.9	7.2	2.47	60.1	0.560	13.7	0.435	10.6		
	S.D.	0.16	5.5	0.74	59.8	1.9	0.49	14.9	0.152	4.3	0.106	3.0		
	n	7	7	7	7	7	7	7	7	7	7	7		
2500	mean	0.43	21.5	5.62	320.3	8.3	2.57	66.1	0.567	14.5	0.422	10.6		
	S.D.	0.18	3.5	1.88	110.3	3.7	1.20	37.0	0.091	4.4	0.098	2.8		
	n	6	6	6	6	6	6	6	6	6	6	6		

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 9 Hepatic Enzyme Activities and Components DEHP Male

Dose (mg/kg)	Total glutathione content			Glutathione S-transferase activity			Glutathione peroxidase activity		
	µmol / g liver	µmol / mg pro / min	Week 66	µmol / g liver / min	µmol / mg pro / min	Week 66	µmol / g pro / min	nmol / g liver / min	Week 66
0	mean	5.83		307.0	2.68		216.2	30.7	
	S.D.	1.33		76.9	0.24		25.2	9.1	
	n	5		5	5		5	5	
100	mean	4.31		250.8	2.41		197.2	27.6	
	S.D.	1.58		72.3	0.47		14.2	4.5	
	n	6		6	6		6	6	
500	mean	3.67		275.1	2.41		201.0	29.9	
	S.D.	1.27		69.3	0.79		22.8	3.3	
	n	7		7	7		7	7	
2500	mean	3.55		246.0	2.23		180.3	26.9	
	S.D.	1.14		54.2	0.41		17.5	3.3	
	n	6		6	6		6	6	

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 9 Hepatic Enzyme Activities and Components DEHP Female

Study No. B000496

Dose (mg/kg)	FAOS U/mg·pro Week 66	CAT U/mg·pro Week 66	CPT U/mg·pro Week 66	Cytochrome P450 content		Testosterone 6β-hydroxylase activity		Lauric acid α-1-hydroxylase activity		Lauric acid m-hydroxylase activity	
				pmol/mg pro Week 66	nmol/g liver Week 66	nmol/mg pro/min Week 66	nmol/g liver/min Week 66	nmol/mg pro/min Week 66	nmol/g liver/min Week 66	nmol/mg pro/min Week 66	nmol/g liver/min Week 66
0	mean	34.8	5.61	183.5	3.3	2.15	37.0	0.384	6.9	0.343	6.1
	S.D.	0.38	21.2	2.76	58.1	1.3	12.0	0.090	2.7	0.071	2.1
	n	6	6	6	6	6	6	6	6	6	6
100	mean	0.40	23.4	3.68	252.0	5.8	48.7	0.442	9.4	0.395	8.5
	S.D.	0.15	5.8	0.60	61.4	3.2	23.3	0.064	2.9	0.100	3.1
	n	6	6	6	6	6	6	6	6	6	6
500	mean	0.96	31.6	4.28	383.5**	10.5**	90.1*	0.773	21.1*	0.469	12.7*
	S.D.	0.86	20.2	2.09	71.0	2.8	21.3	0.336	10.6	0.134	4.2
	n	6	6	6	6	6	6	6	6	6	6
2500	mean	0.55	34.9	6.19	339.6**	7.6	78.7	0.669	14.4	0.452	9.7
	S.D.	0.25	25.4	5.96	19.7	2.1	31.6	0.167	3.3	0.089	1.6
	n	5	5	5	5	5	5	5	5	5	5

Significantly different from control : *, p<0.05 ; **, p<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 9 Hepatic Enzyme Activities and Components DEHP Female

Study No. B000496

Dose (mg/kg)	Total glutathione content			Glutathione S-transferase activity		Glutathione peroxidase activity	
	µmol / g liver	µmol / mg pro / min	µmol / g liver / min	µmol / g pro / min	µmol / g pro / min	nmol / g liver / min	Week 66
0	mean	5.17	2.70	252.3	198.7	25.6	
	S.D.	1.49	0.58	98.6	23.2	6.4	
	n	6	6	6	6	6	
100	mean	4.25	2.94	287.7	215.0	28.7	
	S.D.	1.47	0.67	79.2	22.1	4.8	
	n	6	6	6	6	6	
500	mean	4.02	3.16	336.0	188.0	28.9	
	S.D.	1.17	0.44	50.3	10.0	2.1	
	n	6	6	6	6	6	
2500	mean	4.63	2.66	276.4	203.6	28.1	
	S.D.	1.06	0.71	88.8	18.6	5.1	
	n	5	5	5	5	5	

Significantly different from control : *, P<0.05 ; **, P<0.01.

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 10 Necropsy Findings - Summary
 Scheduled Sacrifice (Week 66)

Organ Findings	Sex		Male		Female	
	Test Substance Dose(mg/kg)	Number of Animals Examined	DEHP	DEHP	DEHP	DEHP
Liver						
Dark brownish			1			
Kidney						
Cyst			1	2	1	1
Testis						
Small			1	2	2	
Epididymis						
Small			2		1	
Seminal vesicle						
Small			2	1	1	
Prostate						
Small			1	1	1	
Ovary						
Prominent corpus luteum					2	2

+: Present; B, Bilateral; U, Unilateral
 N, Finding absent; Y, Finding present

Organ	Findings	Sex		Male DEHP			Female DEHP			
		Dose(mg/kg)	Number of Animals	0	100	500	2500	0	100	500
Spleen				<8>	<9>	<10>	<9>	<6>	<6>	<5>
Liver	Activation, Kupffer cell			<8>	<9>	<10>	<9>	<6>	<6>	<5>
	Extramedullary hematopoiesis			0	0	1	0	0	0	1
	Fatty change, hepatocyte, periportal			1	0	0	0	0	0	0
	Glycogen accumulation, hepatocyte			1	0	0	0	2	1	0
	Inflammatory cell infiltration, focal			1	1	2	3	2	2	2
	Microgranuloma			3	1	2	5	1	1	0
	Necrosis, focal			1	1	2	2	0	0	0
Pancreas	Atrophy, acinus, focal			<8>	<9>	<10>	<9>	<6>	<6>	<5>
	Decrease in zymogen granule			0	0	0	0	0	0	1
	Increase in apoptotic body, acinar cell			1	1	1	1	0	0	0
	Inflammatory cell infiltration, lymphocyte focal			0	0	0	0	1	0	0

<> Number of animals examined

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmoset
 Table 11
 Histological Findings - Summary
 Scheduled Sacrifice (Week 66)

Study No. B000496

Organ Findings	Sex		Male			Female		
	Test Substance Dose(mg/kg)	Number of Animals	DEHP	DEHP	DEHP	DEHP	DEHP	DEHP
Pancreas								
Vacuolation, acinar cell	0	<8>	<9>	<10>	<9>	<6>	<6>	<5>
	8	8	9	10	9	6	6	5
Kidney								
Basophilic tubule, proximal	<8>	<8>	<9>	<10>	<9>	<6>	<6>	<5>
	0	0	1	0	2	2	2	0
Cyst	1	1	3	2	1	2	2	0
Dilatation, Bowman's capsule	2	2	4	4	3	3	0	0
Dilatation, distal tubule	0	0	0	0	0	0	0	1
Extramedullary hematopoiesis	0	0	1	0	0	0	1	0
Fibrosis, focal	0	0	0	0	0	0	0	0
Hemorrhage, renal tubule	0	0	0	0	0	0	0	0
Inflammatory cell infiltration, focal	4	4	7	9	8	5	4	4
Testis, unilateral Growing	<5>	<5>	<6>	<7>	<6>	<6>	<6>	<6>
	0	0	1	1	1	1	1	1
Epididymis, unilateral Growing	<8>	<8>	<9>	<9>	<9>	<9>	<9>	<9>
	0	0	2	1	1	1	1	1

<>, Number of animals examined

Organ Findings	Sex		Male			Female		
	Test Substance Dose(mg/kg)	Number of Animals	DEHP	DEHP	DEHP	DEHP	DEHP	DEHP
Seminal vesicle								
Growing			<8>	<9>	<10>	<9>		
			0	2	1	1		
Prostate								
Growing			<8>	<8>	<9>	<9>		
			0	1	1	1		
Ovary								
Cyst, follicle								
			<6>	<6>	<6>	<6>	<6>	<5>
Cyst, corpus luteum			0	1	0	0	2	0
Uterus								
			<6>	<6>	<6>	<6>	<6>	<5>
Pituitary								
Cyst, anterior lobe			<8>	<9>	<10>	<8>	<6>	<5>
			0	0	1	1	2	1
Hyperplasia, Rathke's pouch, posterior lobe			0	0	0	0	1	0
Inflammatory cell infiltration, lymphocyte, focal			0	0	0	0	0	0

<>, Number of animals examined

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmoset
 Table 11 Histological Findings - Summary Scheduled Sacrifice (Week 66)

Organ Findings	Sex		Male DEHP				Female DEHP			
	Test Substance Dose(mg/kg)	Number of Animals	0	100	500	2500	0	100	500	2500
Thyroid Cyst			<8>	<9>	<10>	<9>	<6>	<6>	<6>	<5>
Ectopic thymic tissue			1	1	1	1	2	1	2	0
Eosinophilic substance, follicular cell, diffuse			0	1	0	0	0	0	0	1
Hyperplasia, follicular cell, focal			0	1	0	0	0	0	0	0
Inflammatory cell infiltration, lymphocyte, focal			1	0	4	0	0	0	0	1
Adrenal Extracapsular cortical tissue			<8>	<9>	<10>	<9>	<6>	<6>	<6>	<4>
Extramedullary hematopoiesis			7	6	8	7	2	4	6	3
Osteous metaplasia			0	0	0	0	0	0	0	1
Pigment deposition, corticomedullary junction			0	1	0	0	0	0	0	0

<> Number of animals examined

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 12 Histological Findings (3 β -hydroxysteroid dehydrogenase immunostaining) DEHP Male

Dose (mg/kg)	(-)	(+)	(++)	(+++)
0	mean 22.50 S.D. 19.29 n 3	43.00 16.93 3	28.67 25.50 3	5.83 7.11 3
100	mean 43.33 S.D. 49.24 n 3	22.00 21.75 3	21.17 18.33 3	13.50 18.43 3
500	mean 45.67 S.D. 43.32 n 3	38.50 28.97 3	14.33 24.83 3	1.50 2.60 3
2500	mean 10.50 S.D. 9.26 n 3	33.33 11.72 3	38.33 7.08 3	17.83 20.97 3

(-), negative; (+), positive 1-30%; (++) , positive 31-80%; (+++), positive 81-100%

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmosets
 Table 12 Histological Findings (3 β -hydroxysteroid dehydrogenase immunostaining) DEHP Female

Dose (mg/kg)	Total Area	Positive Area	%
0	mean	0.59	4.22
	S.D.	1.44	10.35
	n	6	6
100	mean	2.69	13.40
	S.D.	5.77	24.83
	n	6	6
500	mean	8.54	38.10
	S.D.	7.12	28.03
	n	6	6
2500	mean	21.67	22.61
	S.D.	6.37	30.29
	n	5	5

Total Area = Whole ovary area on one section

Positive Area = The area exhibits 3 β -hydroxysteroid dehydrogenase activity on one section

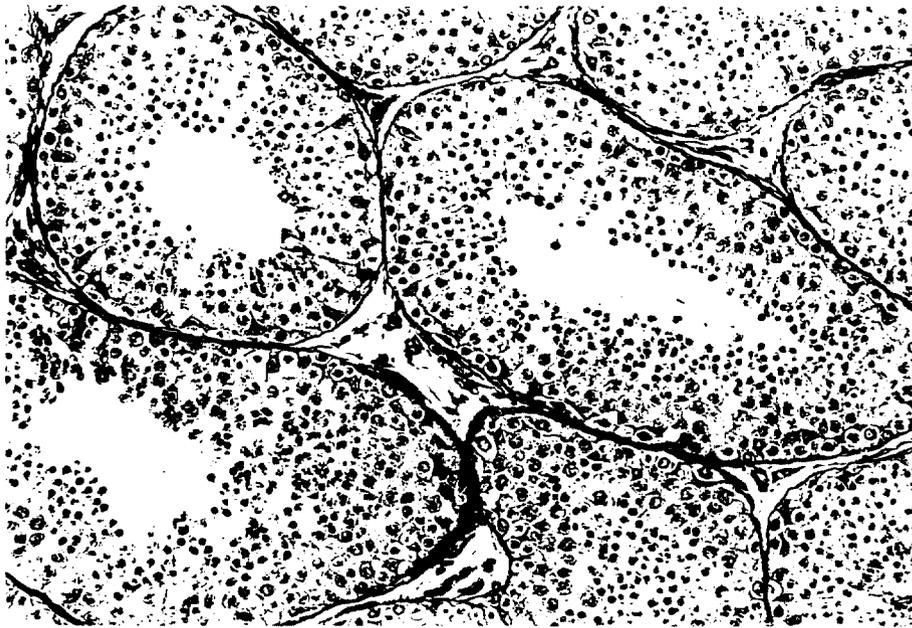
% = (Positive Area/ Total Area) \times 100

Sixty-Five-Week Repeated Oral Dose Toxicity Study of Di(2-ethylhexyl)phthalate(DEHP) in Juvenile Common Marmoset
 Table 1.3 Electron-microscopic Findings Scheduled Sacrifice (Week 66)

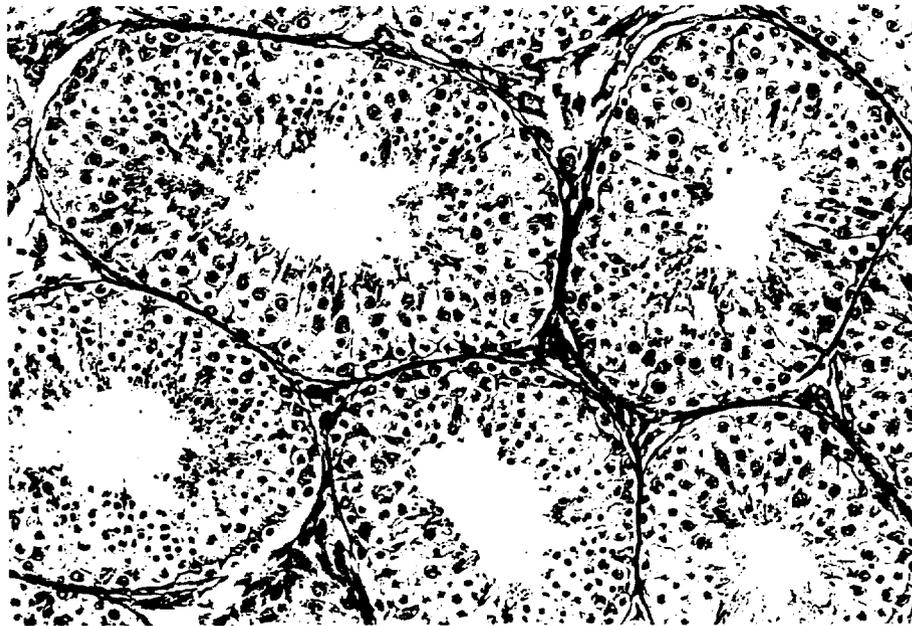
Sex	Test Substance Dose (mg/kg)	DEHP 0	DEHP 100	DEHP 500	DEHP 2500
	Animal No.	1 1 1	1 1 1	1 1 1	1 1 1
		0 0 0	0 0 0	0 0 0	0 0 0
		1 1 1	2 2 2	3 3 3	4 4 4
		0 0 1	0 0 1	0 0 0	0 0 0
Organ Findings		7 8 1	7 8 2	7 8 9	7 8 9
Testis		- - -	- + -	- - -	- - -
Undeveloped spermatogenic cell					
- , Finding absent; + , Finding present					

Photographs 1-12

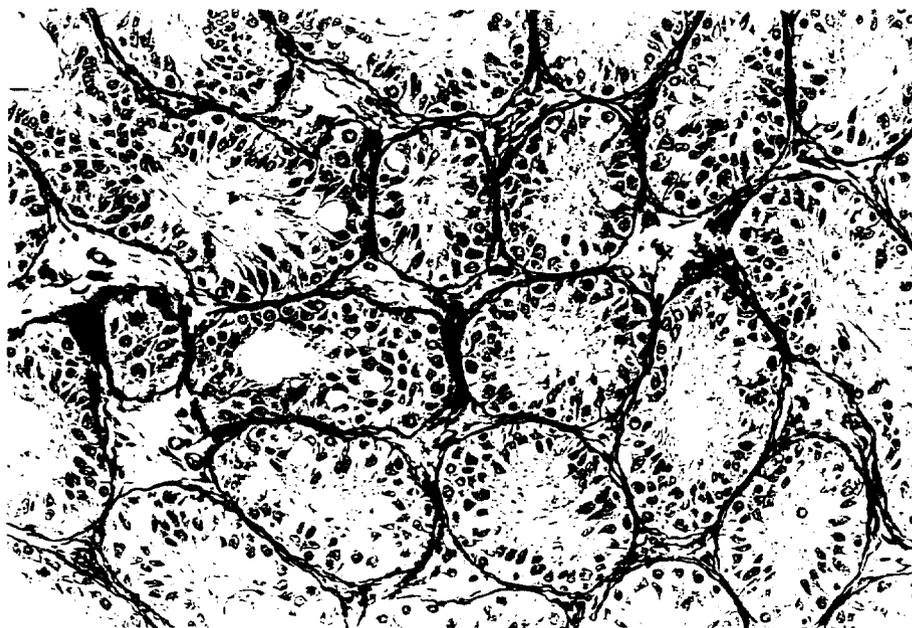
(Study No. B000496)



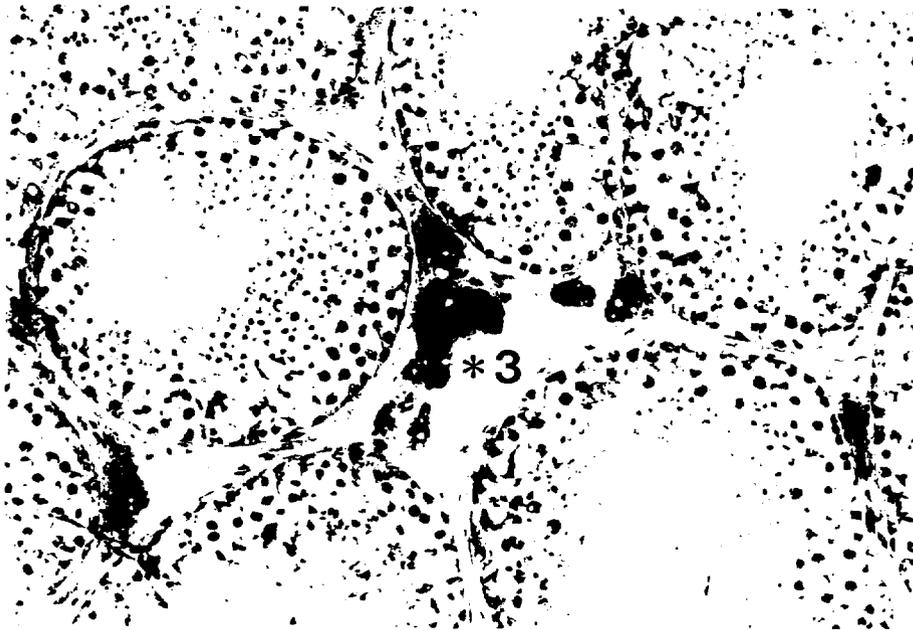
Photograph 1.
Control group, Male, Animal No.10110 (Scheduled sacrifice, Week 66)
Testis: No remarkable change. (HE, x 200)



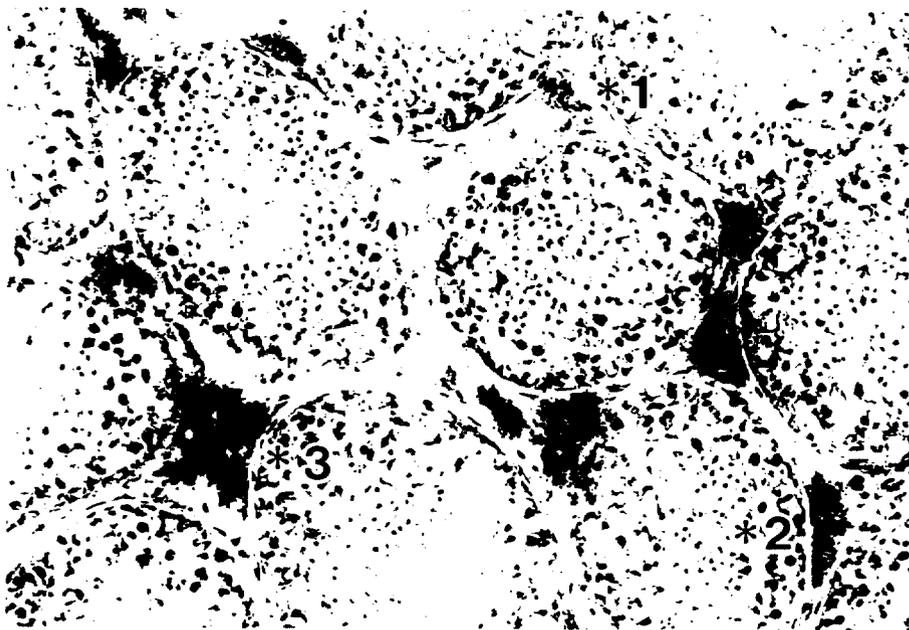
Photograph 2.
DEHP 2500 mg/kg group, Male, Animal No.10404 (Scheduled sacrifice, Week 66)
Testis: No remarkable change. (HE, x 200)



Photograph 3.
DEHP 100 mg/kg group, Male, Animal No.10204 (Scheduled sacrifice, Week 66)
Testis: Growing. (HE, x 200)



Photograph 4.
Control group, Male, Animal No.10111 (Scheduled sacrifice, Week 66)
Testis: 3β -HSD activities are detectable in the Leydig cells. *3 indicates strong activity.
(x 200)



Photograph 5.
DEHP 2500 mg/kg group, Male, Animal No.10409 (Scheduled sacrifice, Week 66)
Testis: 3β -HSD activities are detectable in the Leydig cells. *1, *2 and *3 indicate slight, moderate and strong activities, respectively. (x 200)



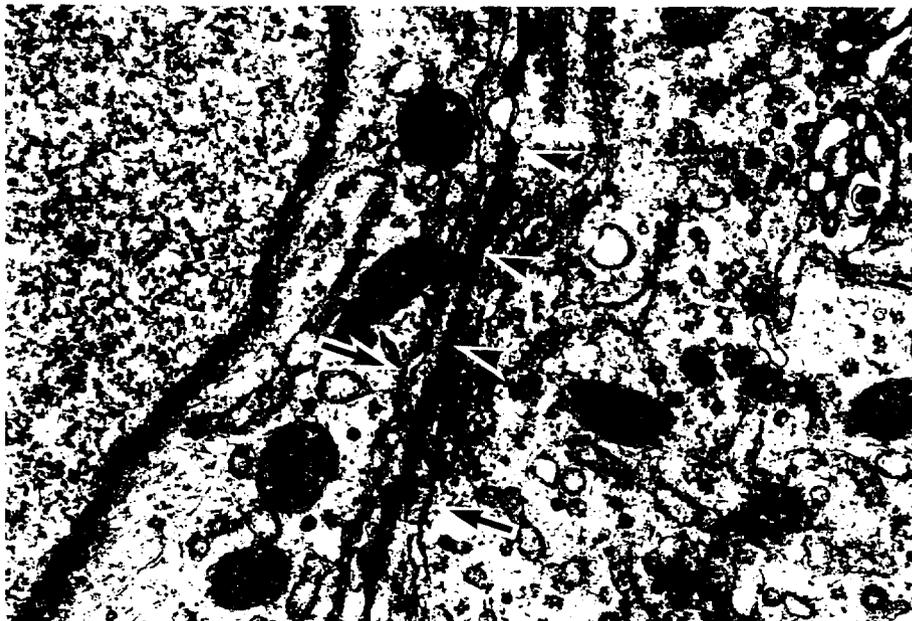
Photograph 6.
DEHP 100 mg/kg group, Male, Animal No.10208 (Scheduled sacrifice, Week 66)
Testis: No 3β -HSD activity is detectable in the Leydig cells. (x 200)



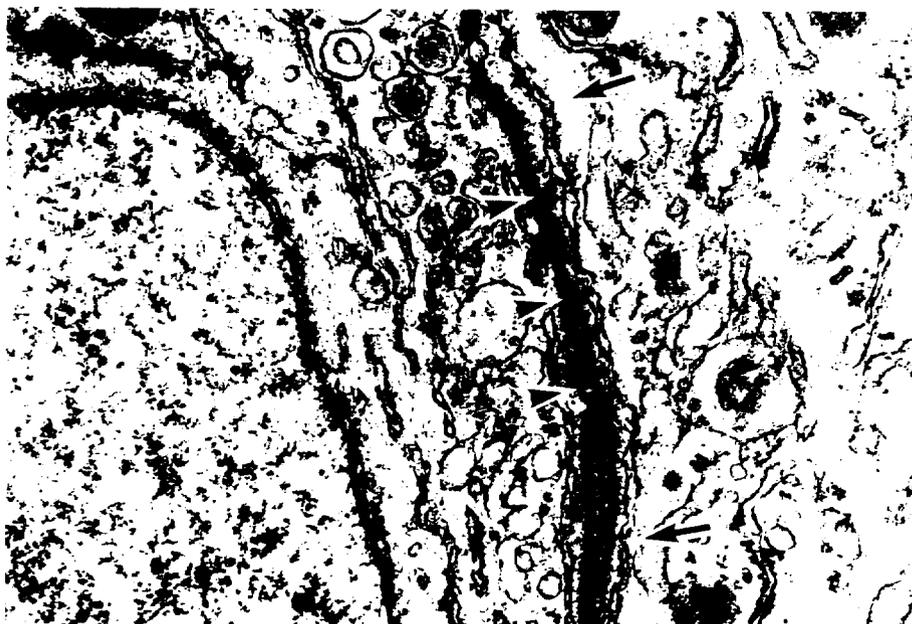
Photograph 7.
Control group, Male, Animal No.10107 (Scheduled sacrifice, Week 66)
Testis: No remarkable change. (Electron micrograph, x 4,050)



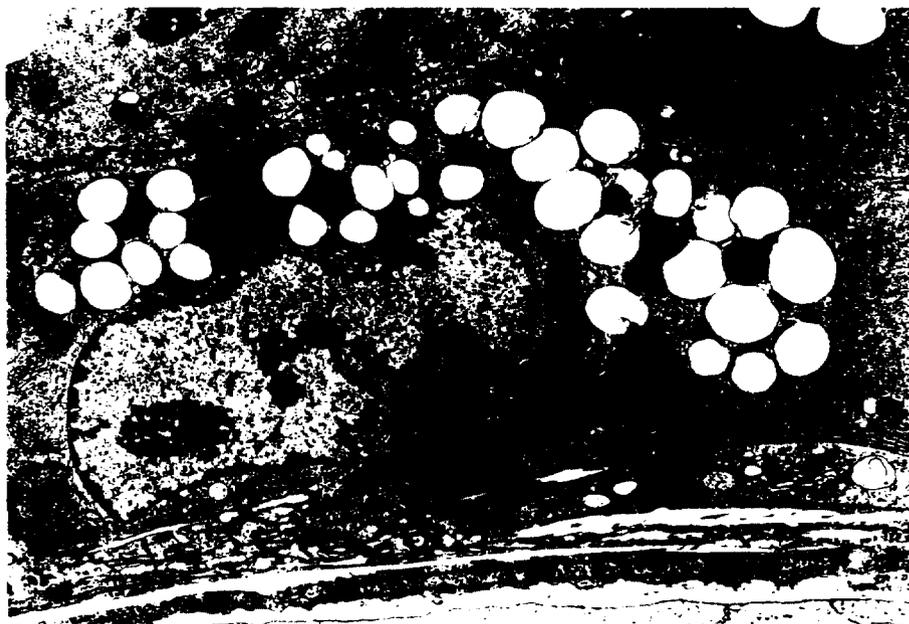
Photograph 8.
DEHP 2500 mg/kg group, Male, Animal No.10408 (Scheduled sacrifice, Week 66)
Testis: No remarkable change. (Electron micrograph, x 4,050)



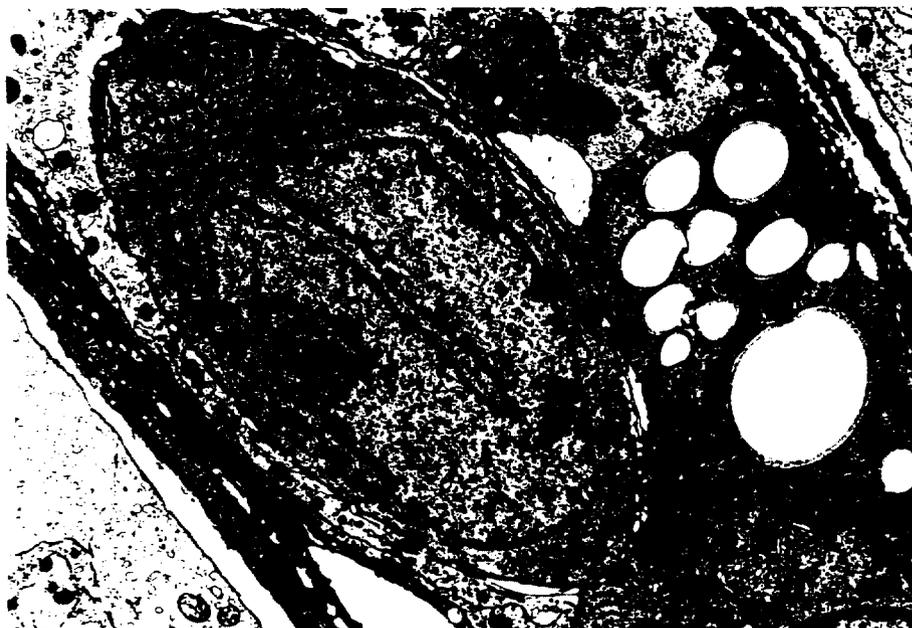
Photograph 9.
 Control group, Male, Animal No.10107 (Scheduled sacrifice, Week 66)
 Testis: No remarkable changes are observed in the junctional complex (arrowheads)
 between the Sertoli cells and rough-endoplasmic reticulums (arrows).
 (Electron micrograph, x 28,000)



Photograph 10.
 DEHP 2500 mg/kg group, Male, Animal No.10408 (Scheduled sacrifice, Week 66)
 Testis: No remarkable changes are observed in the junctional complex (arrowheads)
 between the Sertoli cells and rough-endoplasmic reticulums (arrows).
 (Electron micrograph, x 28,000)



Photograph 11.
Control group, Male, Animal No.10111 (Scheduled sacrifice, Week 66)
Testis: No remarkable changes are observed in the Leydig cell.
(Electron micrograph, x 7,500)



Photograph 12.
DEHP 2500 mg/kg group, Male, Animal No.10407 (Scheduled sacrifice, Week 66)
Testis: No remarkable changes are observed in the Leydig cells.
(Electron micrograph, x 7,500)

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FIGURES

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Table 1-1 Radioactivity Concentrations in Plasma

Time	Concentration of radioactivity (μg equivalent of DEHP/mL)					
	100 mg/kg		2500 mg/kg			
	Male	Female	Male	Female	Male	Female
Group 3 (3 months old)						
1 hr	3.76 \pm 3.17	16.76 \pm 11.23	18 \pm 18			47 \pm 15
2 hr	6.45 \pm 5.56	9.89 \pm 6.65	31 \pm 41			42 \pm 24
4 hr	2.99 \pm 3.28	6.62 \pm 7.17	23 \pm 20			66 \pm 22
8 hr	1.00 \pm 0.53	3.10 \pm 4.48	4 \pm 1			7 \pm 1
12 hr	0.42 \pm 0.39	0.65 \pm 0.61	4 \pm 2			5 \pm 2
24 hr	0.37 \pm 0.28	0.24 \pm 0.16	3 \pm 3			1 \pm 2
48 hr	0.08 \pm 0.07	0.16 \pm 0.07	4 \pm 5			1 \pm 1
72 hr	0.04 \pm 0.04	0.09 \pm 0.05	N.D. ²⁾			N.D. ²⁾
120 hr	0.05 \pm 0.01	0.06 \pm 0.05	N.D. ²⁾			N.D. ²⁾
168 hr	N.D. ¹⁾	N.D. ¹⁾	N.D. ²⁾			N.D. ²⁾

Data are expressed as the mean \pm S.D. of three marmosets.N.D.¹⁾ < 0.04 μg equivalent/mLN.D.²⁾ < 1 μg equivalent/mL

Table 1-2 Radioactivity Concentrations in Plasma

Time	Concentration of radioactivity (μg equivalent of DEHP/mL)					
	100 mg/kg		2500 mg/kg			
	Male	Female	Male	Female	Male	Female
1 hr	10.53 \pm 5.46	19.49 \pm 16.71	50 \pm 39	56 \pm 28		
2 hr	12.10 \pm 7.27	17.65 \pm 17.06	31 \pm 32	51 \pm 48		
4 hr	6.80 \pm 4.00	10.86 \pm 12.07	14 \pm 13	33 \pm 43		
8 hr	2.97 \pm 3.30	4.27 \pm 4.46	8 \pm 6	19 \pm 26		
12 hr	1.93 \pm 1.37	2.78 \pm 2.28	7 \pm 3	12 \pm 16		
24 hr	0.57 \pm 0.31	0.74 \pm 0.62	6 \pm 5	15 \pm 22		
48 hr	0.24 \pm 0.12	0.31 \pm 0.27	2 \pm 0	5 \pm 3		
72 hr	0.18 \pm 0.11	0.27 \pm 0.22	2 \pm 0	3 \pm 3		
120 hr	0.12 \pm 0.10	0.16 \pm 0.16	N.D. ¹⁾	2 \pm 2		
168 hr	0.06 \pm 0.07	0.14 \pm 0.09	N.D. ¹⁾	N.D. ¹⁾		

Data are expressed as the mean \pm S.D. of three marmosets.N.D.¹⁾ < 1 μg equivalent/mL.