NATIONAL INSTITUTE OF PUBLIC HEALTH AND
ENVIRONMENTAL PROTECTION
BILTHOVEN THE NETHERLANDS

Report nr 618902 013

TOXICOLOGICAL INVESTIGATION OF DIBUTYLPHTHALATE IN RATS

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P. de Fluiter and F.X.R. van Leeuwen.

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CONTENTS

SUMMARY

In a study in which male rats have been exposed to 0, 20, 60, 200, 600 and 2000 mg dibutylphthalate (DBP)/kg diet for 2 weeks, body weight and liver weight and a number of enzyme parameters which are related with peroxisome proliferation (palmitoyl coenzyme-A oxidase (PCO), enoyl coenzyme-A hydratase (ECH), carnitine acetyl transferase (CAT) and lauric acid hydroxylase (LAH)) have been determined in liver homogenates.

No effect has been observed on the body and liver weight. For PCO a dose-without-effect was determined of 600 mg BBP/kg diet. The three other enzymes, ECH, CAT and LAH were more sensitive with a dose-without-effect (DWE) of 200 mg BBP/kg diet. Therefore, an overall no-observed-effect-level of 200 mg BBP/kg diet was established in this experiment, corresponding with 19.9 mg BBP/kg b.w./day.

SAMENVATTING

In het hier beschreven onderzoek worden mannelijke ratten blootgesteld aan 0, 20, 60, 200, 600 en 2000 mg dibutyl)ftalaat (DBP)/kg voer gedurende 2 weken. Naast het lichaamsgewicht en het levergewicht is een aantal enzymaktiviteiten zoals palmitoyl coenzym-A oxidase (PCO), enoyl coenzym-A hydratase (ECH), carnitine acetyl transferase (CAT) en laurinezuur hydroxylase (LAH) gemeten die gerelateerd zijn aan de proliferatie van peroxisomen. Geen effect werd gemeten op het lichaams- en levergewicht. Voor PCO werd een dosering-zonder-effect vastgesteld van 600 mg DBP/kg voer. De drie andere enzymen, ECH, CAT en LAH waren gevoeliger met een dosering-zonder-effect van 200 mg DBP/kg voer. Daarom is in dit experiment een algemene dosering-zonder-effect vastgesteld van 200 mg DBP/kg voer, overeenkomend met 19,9 mg DBP/kg b.w./dag.

1. INTRODUCTION

Phthalate esters are widely used as weakeners in plastic materials. The main source of human exposure to these plasticizers is via migration from packaging materials into food. Because a large number of different plasticizers are used, the main goal of this project is to determine the relative toxicity of a number of plasticizers in rats under standardized experimental conditions and to establish no-effect levels for the compounds under investigation based on the same sensitive parameter(s) for peroxisome proliferation. In a report on the toxicity of di(ethylhexyl)phthalate (DEHP) [2] the most sensitive parameters were the morphometric analysis of the number and area of peroxisomes in liver sections, and the enzymatic activities of palmitoyl coenzyme-A oxidase (PCO), enoyl coenzyme-A hydratase (ECH), carnitine acetyl transferase (CAT) and lauric acid hydroxylase (LAH) which have been related to the phenomenon of peroxisome proliferation. Since morphometric analysis is quite laborous only enzyme activities, which appeared to be equally sensitive as morphometric analysis, have been selected as parameters for a comparative toxicity evaluation of various phtalate esters with respect to the potency to proliferate peroxisomes.

In the present report, the dose-response relation of different doses of dibutylphthalate (DBP) on the enzyme induction will be described in a study with male rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Test compound

The test compound dibutylphthalate (DBP; CAS nr. 84-74-2) is a colourless viscous liquid. The compound was obtained commercially from Merck-Schuchardt (Art. nr. 800919, lot 0274154; Darmstadt, Germany), in a glass 1 L bottle at April 04, 1990. The purity of the test substance is described in Section 3.1.

2.1.2 Animals and maintenance

SPF male rats (Wistar Riv:TOX strain) were about 6 weeks of age with an average body

weight of 144 g at the start of the experiment. The animals were weighed at the beginning of the experiment and at day 7 and 14. The animals were numbered individually and housed with two animals in one wire cage. The temperature of the animal housing was measured once a day and varied between 18.5 and 22.5°C. The relative humidity varied between 47 and 56%. An artificial light/dark cyclus of 12 hrs was maintained. The animals had free access to tap water and to a grounded semi-purified diet (SSP-TOX flour). The food consumption was measured twice a week.

2.2 Methods

2.2.1 Experimental procedure

The study has been performed as described in Study plan RIVM Experiment nr. BES-TOX 91-231. In the study 6 male rats (Wistar Riv:Tox strain) per group were exposed for 2 weeks to 0, 20, 60, 200, 600 and 2000 mg DBP/kg diet and 600 mg DEHP/kg diet as a internal control. At the end of day 14 section was performed under light ether anesthesia. After abdominal bleeding the liver was taken from the rats and about 2 g was cut off, weighed and put in a tube containing 8.0 ml Tris buffer (0.1 M, pH 7.4) which was kept on ice. After collection of all tissue samples, the liver was homogenized in a Potter homogenizer and centrifuged for 8 min at 3000 g. The supernatant was stored at -70°C until analysis.

2.2.2 Mixing of DBP in the diet

The mixing of different amounts of DBP in the SSP-TOX standard flour was performed without any carrier.

2.2.3 Methods of analysis of DBP in the diet

The amount of DBP in the diet has been determined by high performance liquid chromatography (HPLC). The extraction with a mixture of methanol and water (75:25, v/v) and the analysis with HPLC have been performed in a similar way as described for DEHP [3]. Di(cyclohexyl)phthalate was used as internal standard.

2.2.4 Enzyme measurements

The enzyme measurements have been described in two RIVM reports 618902 001 [4] and 618902 002 [5]. The principles and measurements of the enzyme activities will be described only briefly in the following sections.

All enzyme determinations have been performed on a centrifugal analyzer (Cobas Bio, Hoffmann-La Roche) except lauric acid hydroxylase which was determined with high-performance liquid chromatograph (HPLC) with fluorescence detection after precolumn derivatization.

The measurements with the centrifugal analyzer were performed as follows. Firstly, the liver homogenate, buffer, and other cofactors are added by centrifugal force. Then the substrate is added and mixed by centrifugal force. The change in extinction is measured between 30 and 120 sec after mixing of the substrate. For PCO the readings have been performed at various time intervals due to a lag phase in the enzymatic reaction at low activities. To obtain a value for the blank reaction, the same procedure is repeated, except that water is added in the second step instead of the substrate. These two measurements are performed threefold in separate runs.

The samples have been stored at -70 C prior and between the analyses of enzyme activities.

2.2.4.1 Palmitoyl CoA oxidase.

With this method the β-oxidation of palmitoyl CoA is determined. After each cycle an acetyl group is cleaved from the substrate to yield acetyl CoA. The activity is measured by the production of NADH. By the addition of cyanide the mitochondrial β-oxidation is inhibited and only the peroxisomale activity is determined. Due to a lag phase in the reaction kinetics at low PCO activities, the reaction has been monitored up to 10 min.

The experimental procedure has been decribed in detail in RIVM-report 618902 001 [4].

2.2.4.2 Enoyl CoA hydratase.

This bifunctional enzyme catalyzes the second and third step in the β-oxidation. The activity is measured by the disappearence (hydratation) of the double bond in the substrate (crotonyl-CoA) at 285 nm. The measurement is performed with and without heat treatment. Since the peroxisomal enzyme appears to be heat labile, whereas the mitochondrial enzyme

is stable upon heat treatment, the difference in activity gives the activity of the peroxisomal enzyme.

The experimental procedure has been decribed in detail in RIVM-report 618902 001 [4].

2.2.4.3 Carnitine acetyl transferase.

This membrane bound enzyme, present in peroxisomes, microsomes and mitochondria, is measured by the release of CoA from acetyl-CoA in the transfer reaction of the acetyl group to carnitine. The sulfhydryl group of the liberated CoA reacts with dithionitrobenzoic acid (DTNB) to yield a yellow-coloured product.

The experimental procedure has been decribed in detail in RIVM-report 618902 002 [5].

2.2.4.4 Lauric acid hydroxylase.

The hydroxylation of lauric acid to 11- and 12-hydroxylauric acid has been determined by HPLC after derivatization of the carboxylic group by 4-bromomethyl-7-methoxycoumarin (BrMMC). The method has been described in detail in RIVM-report 618902 005 [6] (in Dutch) and elsewhere [7] (in English).

2.2.5 Statistics

Statistic analyses have been performed with a t-test for two-sided multi-comparison of the treated groups with the control group, according to Dunnett [8]. The data were applied to a logarithmic transformation after the Bartlett's test for equal variances prior to statistic analysis.

3. RESULTS AND DISCUSSION

3.1 Identity and purity of the test compound

The identification of the test compound has been performed with three independent methods, being Fourier Transform Infra Red Spectroscopy (FTIR), proton and carbon-13 Nuclear Magnetic Resonance (NMR) and HPLC. The detailed data of analysis can be found in addendum I, II and III, respectively. The overall conclusion of the three techniques was that the test compound was indeed DBP. The purity was higher than 99.6%

as determined from HPLC experiments.

3.2 Food intake and calculated intake of DBP.

The food consumption was determined (per two animals) twice a week. The individual data of the food consumption are given in Addendum IV. The intake of DBP by the animals (in mg DBP/kg b.w./day) have been calculated from the food consumption, the body weights and the amount of DBP in the diet as determined by HPLC. The results are shown in Table 1.

3.3 Body and liver weights.

The body and liver weights have been summarized in Table 1. No significant effects in the body and liver weights were observed. The individual data are given in Addendum IV.

3.4 Enzyme measurements.

3.4.1 Palmitoyl CoA oxidation.

The results of the cyanide-insensitve palmitoyl CoA oxidation are shown in Table 2. No ideal dose-response relation was observed. The groups receiving 200 and 2000 mg DCHP/kg diet were statistically significantly different from the control group and not the group receiving 600 mg/kg diet. Because no dose-response relation was observed the dose-without-effect for PCO activity has been established at 2000 mg/kg diet equal to 212 mg/kg b.w./day.

3.4.2 Enoyl CoA hydratase.

The heat labile enzyme, specific for the peroxisomes, was amongst others the most sensitive parameter in this study and shows a dose-related increasing response (Table 2). The groups receiving 600 and 2000 mg DBP/kg diet were statistically significantly different from the control group. The dose-without-effect for ECH activity is 200 mg/kg diet equal to 19.9 mg/kg b.w./day.

3.4.3 Carnitine acetyl transferase.

The activity of the carnitine acetyl transferase showed a clear dose-related increase (Table 2). The enzymatic activity of the groups receiving 600 and 2000 mg DCHP/kg diet was statistically significant different from the control group. The dose-without-effect was therefore 200 mg/kg diet equal to 19.9 mg/kg b.w./day.

3.4.4 Lauric acid hydroxylase.

The activity of this enzyme has been determined by quantitation of products formed in the hydroxylation reaction (11- and 12-hydroxylauric acid). Especially 12-hydroxylase activity showed a dose-related response with significantly different effects in the dose groups receiving 600 and 2000 mg/kg diet (Table 3). The dose-without-effect for the LAH activity was establihed as 200 mg/kg diet equal to 19.9 mg/kg b.w./day.

3.5 No-observed-effect-level

In Table 4, the doses-without-effect of the various parameters. The most sensitive parameter is the increase in activity of the enzymes ECH, CAT and LAH, showing a dose-without-effect of 200 mg/kg diet or 19.9 mg/kg b.w./day. The PCO activity was possibly not less sensitive but no dose-response relation was observed. The overall no-observed-effect-level for DBP in this study has been established as 200 mg DBP/kg diet equal to 19.9 mg DBP/kg b.w./day.

4. CONCLUSIONS

1. A study has been performed with male rats which have been exposed to 0, 20, 60, 200, 600 and 2000 mg dibutylphthalate (DBP)/kg diet for 2 weeks.
2. No effect on body or liver weights has been observed.
3. A dose-effect relationship has been determined for the induction of a number of enzymes involved in peroxisome proliferation, such as palmitoyl coenzyme-A oxidase (PCO), enoyl coenzyme-A hydratase (ECH), carnitine acetyl transferase (CAT) and lauric acid hydroxylase (LAH).
4. Because the PCO activity showed no dose-response relation, a dose-without-effect of 600 mg/kg diet was established. The other enzyme parameters were slightly more sensitive with a dose-without-effect of 200 mg DBP/kg diet.
5. An overall no-observed-effect-level of 200 mg DBP/kg diet was defined corresponding to 19.9 mg DBP/kg b.w./day.

5. ACKNOWLEDGEMENTS

The coworkers of the Central Animal Laboratory (CDL) are acknowledged for the performance of the zootechnical experiments including necropsy. Dr. G. Zomer (BFT) and Mr. T. Visser (LOC) are acknowledged for the NMR and FTIR measurements, respectively.

6. REFERENCES

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J. Liquid Chromatogr., 15, 2247-2260.

8. C.W. Dunnett (1964).
New tables for multiple comparisons with a control.
Biometrics, 20, 482-491.

TABLE 1.

Summary of the mean body and liver weights and the calculated intake of DBP.

dose DBP mg/kg diet	liver weight(g)		body weight(g)		intake DBP (mg kg b.w/day)
	mean	sd	mean	sd	
0	9.35	0.51	225.2	13.7	0
20	9.02	0.89	221.3	13.5	1.1
60	9.17	0.52	224.2	13.1	5.2
200	9.24	0.98	225.7	16.0	19.9
600	9.25	0.97	226.5	13.4	60.6
2000	9.87	0.37	226.3	11.9	212.5
600 DEHP	9.85	0.49	223.8	11.5	46.2

* $p < 0.05$; ** $p < 0.01$.

TABLE 2.

Summary of the mean enzyme activities and statistical analysis of palmitoyl CoA oxidase (PCO), enoyl CoA hydratase (ECH) and carnitine acetyl transferase (CAT).

dose DBP mg/kg diet	PCO U/g protein mean	sd	ECH kU/g protein mean	sd	CAT U/g protein mean	sd
0	2.83	0.47	0.74	0.20	0.87	0.21
20	2.97	0.41	0.86	0.06	0.82	0.52
60	2.96	0.24	0.95	0.11	0.96	0.42
200	3.70	0.47 *	0.86	0.09	1.31	0.62
600	3.46	0.58	0.99	0.17 *	2.12	0.57 **
2000	5.79	0.87 **	2.99	0.90 **	11.20	2.79 **
517 DEHP	5.07	0.70 **	2.17	0.15 **	9.22	1.36 **

* $p < 0.05$; ** $p < 0.01$.

TABLE 3.

Summary of the enzyme activities and statistical analysis of 11- and 12-lauric acid hydroxylase (cytochrome P-450 IVA1).

dose DBP mg/kg diet	activity (U/g protein)	
	11OH	12OH
0	0.21 ± 0.03	0.23 ± 0.06
20	0.21 ± 0.04	0.24 ± 0.06
60	0.25 ± 0.03	0.31 ± 0.06
200	0.23 ± 0.05	0.28 ± 0.05
600	0.28 ± 0.06 *	0.38 ± 0.09 **
2000	0.67 ± 0.17 **	1.58 ± 0.45 **
600 DEHP	0.64 ± 0.06 **	1.45 ± 0.23 **

* $p < 0.05$; ** $p < 0.01$.

TABLE 4.

Summary of doses-without-effect of several parameters in the present DBP study.

parameter	dose-without-effect	
	mg/kg diet	mg/kg bw
body weight	>2000	>213
liver weight	>2000	>213
palmitoyl CoA oxidation	600	60.6
enoyl CoA hydratase	200	19.9
carnitine acetyl transferase	200	19.9
lauric acid hydroxylase	200	19.9

ADDENDUM I (report 618902 013)

FTIR identification data of DBP.

Page 1 and 2: general information and results.
Page 3: FTIR spectrum of DBP.

rivm
onderzoek in dienst
van mens en milieu
RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEUHYGIENE

RAPPORTAGE ANALYSERESULTATEN Infraroodspectrometrie
Laboratorium voor Organisch-analytische Chemie, Afdeling Molecuulspectrometrie

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Administratieve gegevens

- *opdrachtgever*

Naam	:P.de Fluiter
Instelling	:RIVM
Lab/afdeling	:TOX
Gebouw/kamernummer	:A3006
Adres	:
Postbak	:0
Telefoonnummer	:tst 2744

- *monster*

Projectnummer	:618902 (631401)
Projectnaam	:Ftalaten
Datum ontvangst	:21-05-1991
Monsterinformatie	:standaarden

- *opdrachtnemer*

Registratieformulier	:920098
MS-nummer(s)	:92-687 t/m 92-694
Datum analyse	:12-06-1992
Uitgevoerd door	:T.Visser
Onderzoeksleider	:T.Visser
Paraaf voor akkoord	:

Vraagstelling.

Kwaliteitscontrole van standaardchemicaliën

Werkwijze.

De vloeibare monsters zijn geprepareerd alls vloeistoffilm volgens SOP LOC/215. De kristallijne monsters zijn geprepareerd als vaste stof volgens de KBr-tablet methode (SOP LOC/217/00). De opgenomen spectra zijn basislijn gecorrigeerd en genormeerd volgens SOP LOC/162/00.
Vervolgens is de identiteit van de monsters gecontroleerd aan de hand van de criteria gesteld in SOP LOC/220/01.

Meetgegevens.

Instrument: Bruker IFS-85 FTIR. Detector DTGS, resolutie 2 cm^{-1}, 32 scans.
Sampling technieken: KBr-tablet (referentie KBr),en demontabele cel (referentie purged air).IR-filenummer: IR2733 t/m IR2740.
Opslag: floppy B68

Resultaten.

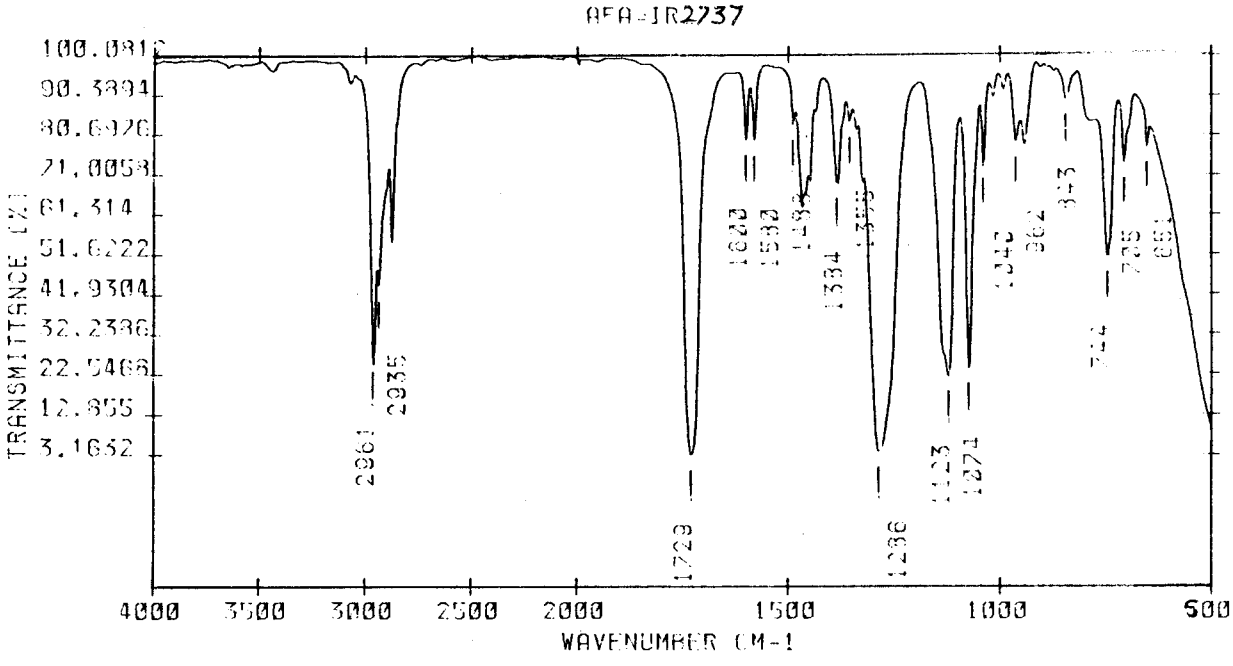
De volgende monsters zijn geanalyseerd:

monster	batchnr	MS nr.	IR-nr	ref.spectrum
diethylhexyl-ftalaat	01	92-687	IR2733	DMS 4750
diisodecyl-ftalaat	02	92-688	IR2734	Sadt.7466*
diisononylftalaat	03	92-689	IR2735	DMS 3202
dicyclohexyl-ftalaat	04	92-590	IR2736	Sadt.674*
dibutyl-ftalaat	05	92-591	IR2737	DMS 2798
diethyl-ftalaat	06	92-592	IR2738	DMS 2797
benzylbutyl-ftalaat	07	92-593	IR2739	Sadt.1837*
bis-2-ethylhexyl-adipaat	08	92-594	IR2740	Sadt.24*

Alle aangeboden monsters voldoen aan de identiteitscriteria gesteld in SOP LOC/220/01. De gevolgde "routing" in dee SOP is voor alle spectra 6.1->6.4->6.7->6.9->6.10->6.21.
De spectra zijn derhalve in overeenstemming met de voorgestelde struktuur. Er zijn geen verontreinigingen zichtbaar in de spectra.

* Collectie Rijksuniversiteit Utrecht.

bijlage : spectra Ir2733 t/m IR2740



SAMPLE : DIBUTYLETALAAT BATCHNR.: 02/4154(05)
ORIGIN : RIVM/TOX(F.DE FLUITER) TECHNIQUE :FILM DATE 12/08/92
SCANS : 32 RESOLUTION : 2.0 /CM VEL : 0
REMARKS : BRUTOFORMULE: C16H2204
OPERATOR : T.VISSER LOC-MS-92-091 ANALYSEOPDRACHTNR.: 920008

ADDENDUM II (report 618902 013)

NMR identification data of DBP.

Page 1 and 2: general information and results.
Page 3: 1H-NMR spectrum of DBP.
Page 4: 13C-NMR spectrum of DBP.

BFT-NMR briefnr. : 92/003mj
verzenddatum : 920617
projectnr. : 618902

Resultatenrapport NMR-spectroscopie (BFT-NMR)

Administratieve gegevens

Uw monster met kenmerk . . . . . :001 t/m 008
Naam opdrachtgever. . . . . :Petra de Fluiter tel.nr.:2714
Datum ontvangst . . . . . :920311
Ons kenmerk (monstercode) . . . . . :PF001/PF008
NMR-analyse code (spectrumcode). . :92mj27b/i, 92mj271a,92mj27b1/h1
Uitgevoerd door . . . . . :Marjorie Jacquemijns
Datum analyse . . . . . :maart-juni 1992 Paraaf:
Akkoord d.d. . . . . :920617 Paraaf:

Instrumentele conditie

Instrument. . . . . : JEOL GSX270
Oplosmiddel. . . . . : aceton D_6
Temperatuur. . . . . ^{1}H-spectra: 20°C, ^{13}C-spectra: 27°C
Kern . . . . . : ^{1}H en ^{13}C
Techniek(en) . . . . . :1D

Vraagstelling. . . . . : structuurconfirmatie

Resultaten (zie eventuele bijlagen)
Opdrachtenformulier RIVM-BFT NMR-Spectrometrie: zie bijlage 1
^{1}H-spectra: zie bijlagen 2, 4, 6, 8, 10, 12, 14, 16
^{13}C-spectra: zie bijlagen 3, 5, 7, 9, 11, 13, 15, 17

Conclusies

De structuur van de aangeboden stoffen 001, 004/008 wordt door NMR bevestigd. De monsters 002 en 003 zijn inderdaad isomeermengsels maar mogen op grond van het feit dat er volgens NMR geen duidelijk hoofdproduct aanwezig is niet als diisononyl- en diisodecylftalaat benoemd worden doch slechts als bis(C9)- en bis(C10)ftalalaat.

De aangegeven zuiverheid van >90% wordt door de NMR-spectra bevestigd.

033

volgnummer :.....

Opdrachtenformulier RIVM-BFT NMR-Spectrometrie

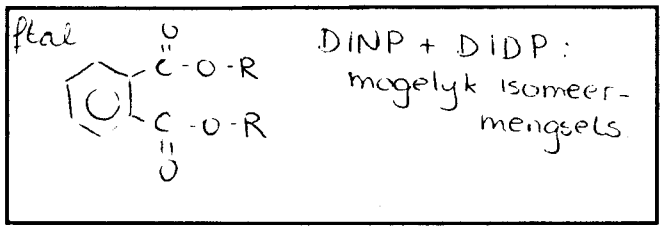
2e sopne. 071 nalf

☞ **Gegevens opdrachtgever:**

Naam	:Pedro de Fluiter	Datum	:110392
Lab / Afd	:Tox	Gebouw / kamernr	:A3086
Projectnaam	:ftalaten	Projectnr	:618902
Paraaf inzender	:PF	Telefoon	:2714

☞ **Gegevens monster:**

Naam	:ftalaten + 1 add part	Bewaarcondities	:kamertemp
Monstercode	:1 % m/m		
Structuurformule	:		



Herkomst :Merck + Exxon Chem Zuiverheid :>99%

☞ **Gegevens analyse** (aankruisen resp. invullen):

Oplosmiddel:

$CDCl_3$	Ander oplosmiddel: Motivering: ivm oplosbaarheid

Kern:

~~^{1}H~~	~~^{13}C~~	^{31}P	^{19}F	Andere:

Techniek:

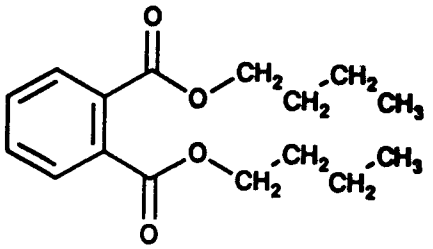
1D	2D	COSY NOESY	Andere techniek: Motivering:

Meettemperatuur (alleen invullen indien afwijkend van kamertemperatuur):.....°C
Doel van de meting (evt vervolgen op de achterkant):..structuurconfirmatie
Monster retour : neen / ja
Bewaren ruwe data : ~~neen~~ / ja tot .....

☞ **Gegevens NMR-spectrometrie:**

Paraaf voor ontvangst	:.....	Datum ontvangst	:110393
NMR-analyse door	:....	Datum analyse	:2e spectra
		Datum monster retour	:.....
Ruwe data	:93nj27 B 19 A1 M1	Datum rapportage	:920616
Gezien paraaf	:.....	Datum afgewerkt	:920617

PF005



dibutylftalaat

21-MAY-92 11:24:13

Acquisition

EXMOD	SGNON
OBNUC	1H
OBFIN	5400.0 Hz
MENUF	J1H
POINT	16384
FREQU	5405.4 Hz
SCANS	16
ACQTM	1.516 sec
PD	1.000 sec
PW1	3.3 us
IRATN	0

Sample

DFILE	92MJ27F
TEMP.	20.0 c
SLVNT	ACETN

21

Ftf

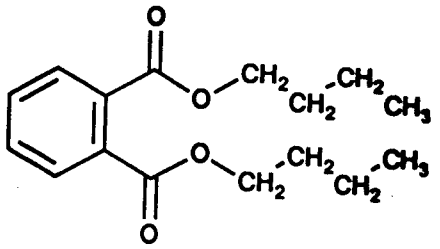
BF	0.10 Hz

Plotting

EXREF	2.05 ppm
XE	4000.0000 Hz
XS	-500.0000 Hz
YG	2.05

Operator

PF005



dibutylftalaat

15-JUN-92 14: 49: 49
DFILE 92MJ27E1
COMNT PF005
EXMOD BCM
OBNUC 13C
OBFIN 5600.0 Hz
POINT 32768
FREQU 17006.8 Hz
SCANS 16
ACQTM 0.963 sec
PD 2.037 sec
PW1 3.3 us
IRFIN 5400.0 Hz
IRATN 0
IRRPW 50 us
TEMP. 27.0 c
SLVNT ACETN
EXREF 30.30 ppm
BF 1.00 Hz
RGAIN 27
XE 17006.8000 Hz
XS 0.0000 Hz

ADDENDUM III (report 618902 013)

HPLC analysis data of DBP.

Page 1: general information and analysis report
Page 2: HPLC chromatogram of DBP.

AREA/PERCENT REPORT

*****SAMPLE ID***** COLUMN: [chromsph C18, 107648]
* standaard 650ul * MEMO: [0-1,60% 1.1-9,70%]
* VIAL #: 0 * MEMO: [9.1-15,95% 16,60% 46]
****

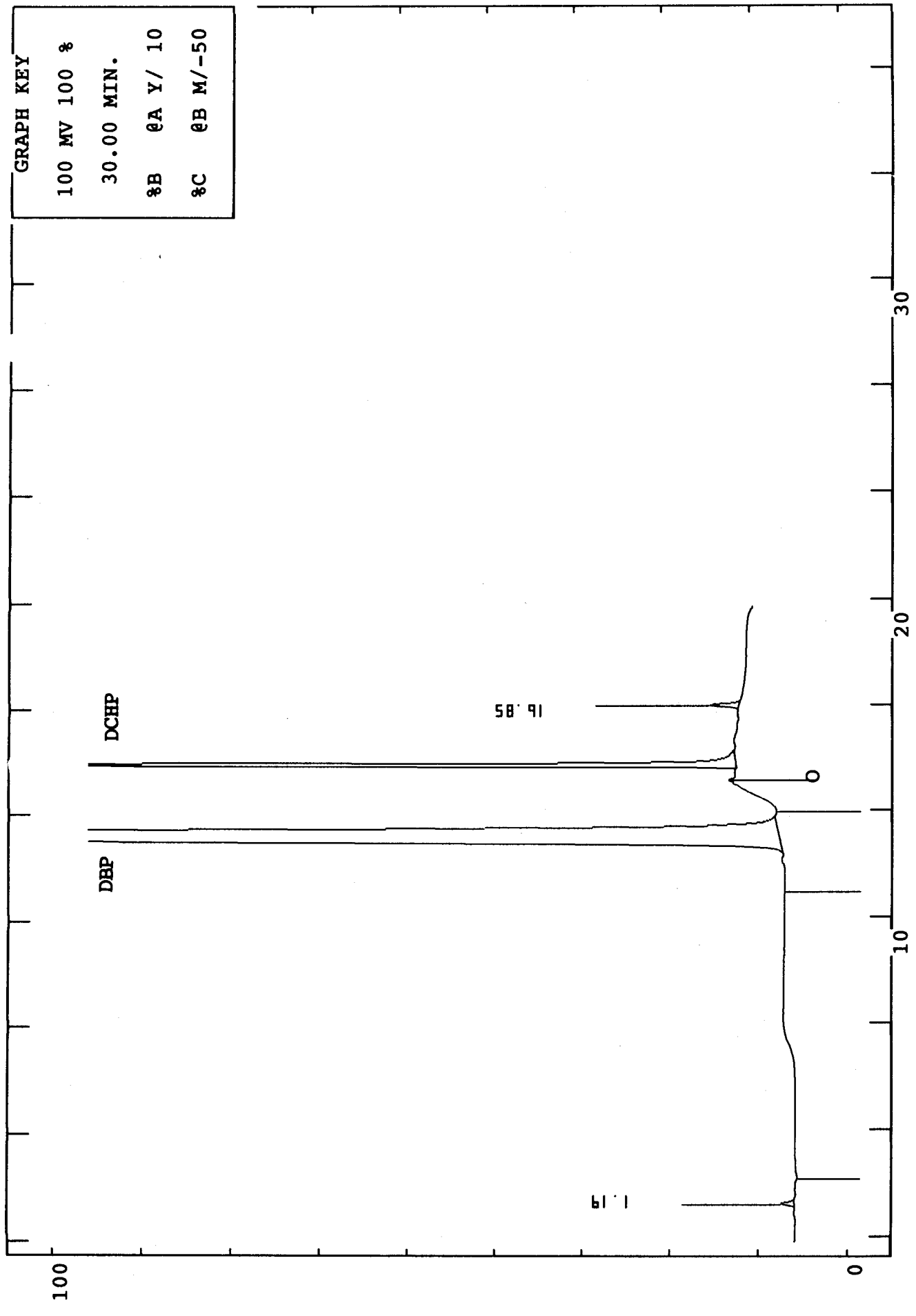
METHOD: [1] [ftalaten, deel1, DBP]
INTEGRATION: [1] [ftalaten, deel1, DBP]
SEQUENCE: [1] [ftalaten, deel1, DBP]

SIGNAL ACQUISITION FROM [MIN] TO [20.00 MIN]

IS AMT: [ ] WGT: [ ] DIL FAC: [ ]
CONVERT FACTOR: [ ]

RETEN.	COMPOUND	AREA	AREA%	HGHT
1.19		156	0.13%	1552
12.66	DBP	101779	87.12%	366766
14.93	DCHP	14497	12.40%	182777
16.85		390	0.33%	3644
TOTALS:		116822	100.00%	

SEQ 6 INJ 1/ 1 SYSTEM[1]
DATA FILE: 2402uv.061
ORIG. 14:51-- 02/24/1992
CURR. 08:34-- 02/25/1992


GRAPH KEY
100 MV 100 %
30.00 MIN.
%B @A Y/ 10
%C @B M/-50
100
0
30
20
10
DCHP
DBP
16.85
1.19

ADDENDUM VI (report 618902 013)

Individual animal and enzyme data.

Page 1: individual body and liver weights (g) and feed consumption per two animals (g).
Page 2: protein contents and enzyme activities of the individual liver homogenates.

DBP-experiment:

Animal number	Group	Body weight Time0 31/10	Week1 07/11	Week2 14/11	Liver weight 14/11	Feed-consumption 31/10- 03/11	04/11- 06/11	07/11- 10/11	11/11- 13/11
2727	1	127	162	202	9.3	119	98	143	110
2728	1	141	186	236	10.0				
2729	1	157	197	234	9.5	129	104	143	105
2730	1	145	179	219	8.9				
2731	1	156	199	238	9.8	126	98	135	102
2732	1	146	184	222	8.7				
2733	2	142	184	228	9.5	120	93	128	98
2734	2	142	180	213	8.3				
2735	2	146	188	228	9.0	120	94	130	98
2736	2	129	164	199	7.7				
2737	2	146	182	223	9.8	128	105	147	113
2738	2	149	195	237	9.9				
2739	3	150	191	236	10.0	124	102	149	114
2740	3	130	166	201	8.9				
2741	3	150	193	234	9.0	131	98	142	106
2742	3	156	191	227	8.4				
2743	3	136	174	218	9.4	122	103	146	104
2744	3	150	192	230	9.3				
2745	4	152	187	214	8.1	122	100	133	97
2746	4	138	172	204	8.1				
2747	4	147	196	245	10.7	133	109	153	113
2748	4	160	201	241	9.7				
2749	4	139	181	231	9.3	117	99	149	112
2750	4	136	175	219	9.5				
2751	5	134	174	222	9.6	118	93	136	105
2752	5	148	183	222	8.9				
2753	5	143	180	210	7.8	112	90	128	94
2754	5	145	183	222	8.8				
2755	5	145	187	234	9.9	140	116	157	119
2756	5	157	203	249	10.5				
2757	6	144	181	218	9.4	119	98	138	103
2758	6	143	183	221	9.5				
2759	6	139	174	216	9.9	130	106	151	114
2760	6	152	193	236	10.2				
2761	6	153	204	246	10.4	124	104	151	114
2762	6	127	170	221	9.9				
2763	7	152	196	238	10.6	134	101	138	101
2764	7	142	185	222	9.6				
2765	7	138	176	212	9.4	113	95	133	99
2766	7	138	177	215	9.9				
2767	7	140	179	218	9.4	125	97	140	106
2768	7	153	193	238	10.3				

DBP-experiment:

Animal number	Group	Protein g/l	PCO U/l	ECH k.U/l	LAH-11 U/l	LAH-12 U/l	CAT U/l
2727	1	21.6	53	11	3.8	3.7	20
2728	1	23.2	64	21	4.1	4.9	15
2729	1	22.6	74	18	5.7	7.8	19
2730	1	22.8	80	23	5.1	5.9	23
2731	1	22.9	58	13	4.6	4.4	26
2732	1	21.8	52	13	4.7	4.3	13
2733	2	24.3	78	21	5.0	4.8	24
2734	2	21.4	70	19	5.2	6.6	25
2735	2	22.2	66	19	4.7	6.1	16
2736	2	22.3	67	21	6.0	6.5	33
2737	2	21.5	47	16	3.6	3.7	12
2738	2	22.1	70	19	4.3	4.2	0
2739	3	23.0	78	21	4.7	4.7	19
2740	3	20.5	63	21	5.0	6.2	10
2741	3	20.7	61	20	5.2	6.6	12
2742	3	22.3	62	17	5.4	6.7	20
2743	3	19.5	57	21	5.8	7.5	29
2744	3	21.0	57	19	6.0	7.2	30
2745	4	22.8	76	17	5.3	5.4	13
2746	4	20.4	84	17	6.6	7.0	18
2747	4	19.4	69	19	3.8	4.9	22
2748	4	22.4	67	19	4.9	5.9	30
2749	4	21.6	88	20	3.9	5.2	33
2750	4	20.4	84	17	4.3	6.7	49
2751	5	21.7	77	22	5.7	8.9	50
2752	5	21.5	66	15	5.2	7.8	38
2753	5	20.6	72	20	5.8	6.3	32
2754	5	21.2	63	23	8.3	11.2	35
2755	5	20.8	94	24	5.6	8.6	63
2756	5	21.5	68	23	5.2	6.0	52
2757	6	21.2	133	53	12.9	30.7	233
2758	6	18.6	131	46	15.8	34.7	174
2759	6	21.6	108	81	9.9	21.4	295
2760	6	20.5	95	43	10.6	23.7	164
2761	6	19.3	113	52	16.4	41.1	189
2762	6	19.9	118	88	14.8	37.1	305
2763	7	21.5	115	45	15.4	38.0	200
2764	7	19.5	106	42	12.0	25.9	185
2765	7	19.5	116	45	13.5	32.8	172
2766	7	19.1	91	45	12.0	26.1	205
2767	7	21.3	83	41	12.1	25.5	145
2768	7	20.4	103	44	12.8	27.3	207