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The Toxicity of Tetrachlorobenzyltoluenes (Ugilec 141) and Polychlorobiphenyls (Aroclor 1254 and PCB-77) Compared in Ah-responsive and Ah-nonresponsive Mice

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ABSTRACT

The toxicity of the PCB substitute Ugilec 141, a mixture of tetrachlorobenzyltoluenes (TCBTs), is compared with the toxicity of a commercial mixture of polychlorobiphenyls (Aroclor 1254) and with the model toxic PCB-congener 3,3',4,4',-tetrachlorobiphenyl (PCB-77) as a positive control. Alterations in liver weight, hepatic cytochrome P450 content and EROD and PROD activity, plasma thyroxin and retinol level, hepatic retinoid level and liver and thyroid pathology, have been studied in Ah-responsive and Ah-nonresponsive mice. Ugilec 141 proved to induce similar toxicological changes, qualitatively and quantitatively, to Aroclor 1254. Therefore Ugilec may pose a similar environmental and health risk as PCBs.

The criteria for acceptance of new substances, like Ugilec 141, on the European market are discussed.

INTRODUCTION

PCBs have been widely used due to their chemical stability, their good thermal conductivity and their dielectric properties. As a consequence of their chemical stability, they belong to the most ubiquitous and persistent xenobiotics in the environment. Nowadays in most western countries, the use of PCBs is prohibited, except for refill of already existing closed systems.

Different PCB substitutes have been suggested, including polyglycol and

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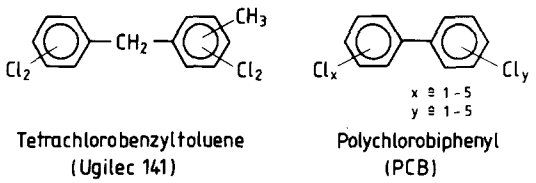

Fig. 1. Structure of tetrachlorobenzyltoluene (TCBT, Ugilec 141) and polychlorobiphenyl (PCB).

tetrachlorobenzyltoluenes (TCBTs) (Peter *et al.*, 1989). The commercial product Ugilec 141, a mixture of tetrachlorobenzyltoluene isomers (TCBTs), has good physical properties, which are comparable to those of PCBs. It is used as a substitute for PCBs in hydraulic liquids which are inflammable, destined for industries, especially mines, as a dielectric fluid for capacitors, and as a cooling and isolation fluid for transformers (Poppe *et al.*, 1988). In Germany Ugilec 141 has been commonly used for hydraulic machinery in underground mining since 1985. In 1987 the estimated loss of Ugilec 141 from these mines amounted to 700 t (Poppe *et al.*, 1988).

TCBTs accumulate in fish and sediment to a similar extent to PCBs (Friege *et al.*, 1989). In Western Germany levels up to 25 mg kg^{-1} Ugilec 141 in the edible part of fish caught in rivers passing through areas with extensive mining have been recorded (Fürst *et al.*, 1987). Although TCBTs were never used in the Netherlands, levels up to 4·8 mg kg^{-1} have been found in red eel from the Dutch river Roer at the Dutch/German border (Wester & van der Valk, 1990).

Up till now, only the acute aquatic toxicity of Ugilec 141 has been tested, which proved to be the same as the acute toxicity of the commercial PCB mixtures Clophen A 30 (Poppe *et al.*, 1988). Based on the remarkable structural resemblance between TCBTs and PCBs (Fig. 1), PCB-like toxicity was expected. This is partly Ah-mediated, such as thymus atrophy, hepatomegaly, involving induction of cytochrome P450 isozymes (Goldstein & Safe, 1989), and partly not Ah-mediated, such as plasma retinol and thyroxin reduction (Brouwer & van den Berg, 1986; Brouwer, 1989). This prompted us to perform a comparative toxicity study between Ugilec 141 and Aroclor 1254 in Ah-responsive (C57BL/6) and Ah-nonresponsive (DBA/2) mice. This study was mainly focused on the characteristic toxic effects mentioned before. The model toxic PCB-congener 3,3′,4,4′,-tetrachlorobiphenyl (PCB-77) was used as a positive control.

MATERIALS AND METHODS

Female Ah-responsive C57BL/6/OLA/HSD and Ah-nonresponsive DBA/2/OLA/HSD mice were used at the age of 10 weeks. The mice were housed, six per cage, in polyethylene cages on metal grids in a separate room.

The mice were supplied with standard laboratory fodder (AM II Hope farms, Woerden, The Netherlands) and water *ad libitum*. The mice were randomly divided in groups of six mice of each strain, and intraperitoneally injected with one of the following dosages per kilogram bodyweight: Ugilec 141 (Prochem, Wessel, West Germany) at 50 mg kg^{-1} and 200 mg kg^{-1}; Aroclor 1254 (Analabs Inc. No. Haven, Conn., USA) at 50 mg kg^{-1} and 200 mg kg^{-1}; PCB-77 (Chrompack, Middelburg, The Netherlands) at 50 mg kg^{-1}. Control groups were exposed to the vehicle corn oil (5 ml kg^{-1}).

Blood was collected at day 1 and day 4 after exposure, by tailbleeding under ether anaesthesia. At day 7, the mice were sacrificed and blood, liver and thyroid glands were collected for biochemical analyses and histopathological examination. Liver and body weight were recorded immediately.

Blood was centrifuged at 1000 *g* and plasma was stored at −20°C. All tissues were immediately frozen in liquid nitrogen and stored at −20°C until further analysis. Tissues for histopathological examination were fixed in 5% formaldehyde.

In the liver, the following parameters were determined: microsomal cytochrome P450 content, ethoxyresorufin-*o*-deethylase (EROD) and pentoxyresorufin-*o*-deethylase (PROD) activity, and retinoid levels in whole liver homogenates. Retinol and total thyroxin (TT4) levels were determined in the plasma.

Extraction and HPLC analysis of hepatic and plasma retinoid was performed according to Brouwer & van den Berg (1986). Total thyroxin (TT4) concentrations were determined in 10 µl aliquots of mouse plasma by chemiluminescence immunoassay, using commercially available kits (Amerlite TT4 assay, Amersham Internat. plc., Amersham, UK). Preparation of hepatic microsomes, total cytochrome P450 measurements (Omura & Sato, 1964), EROD and PROD (Prough *et al.*, 1978; Lubet *et al.*, 1985) assays were performed according to previously published methods.

Statistical analysis was performed using Student's *t*-test. Statistical significance was set at the $p < 0{\cdot}05$ level.

For light microscopic histopathological examination, formalin-fixed slides were stained with Haematoxilin/Eosin according to standard procedures.

RESULTS

Body and liver weight

The liver weight of the Ah-responsive C57BL/6 mice was significantly increased in the high dose Ugilec 141 group (to 141% of the cornoil-treated

TABLE 1
Effects of Ugilec 141, Aroclor 1254 and PCB-77 on Four Hepatic Toxicity Parameters in Ah-Responsive (C57BL/6) and Ah-Nonresponsive (DBA/2) Mice

Mouse strain treatment	*Liver weight*[a] *(g)*	*Cyt. P450 (μmol per liver)*	*EROD*[b] *(nmol min*$^{-1}$*)*	*PROD*[b] *(nmol min*$^{-1}$*)*
C57BL/6				
Cornoil	0·88 ± 0·10	1·35 ± 0·28	6·5 ± 1·7	1·1 ± 0·7
Ugilec 141				
50 mg kg^{-1}	1·00 ± 0·11	1·81* ± 0·23	8·9* ± 1·5	1·3 ± 0·3
200 mg kg^{-1}	1·24* ± 0·13	2·23* ± 0·28	16·3* ± 15·4	1·8 ± 1·2
Aroclor 1254				
50 mg kg^{-1}	1·07* ± 0·08	1·67 ± 4·19	28·6 ± 33·5	4·0* ± 2·2
200 mg kg^{-1}	1·17* ± 0·04	3·60* ± 1·30	54·8* ± 27·1	16·5* ± 9·2
PCB-77				
50 mg kg^{-1}	1·04* ± 0·12	2·23 ± 1·27	230·8* ± 186·2	6·7* ± 3·5
DBA/2				
Cornoil	1·13 ± 0·21	1·17 ± 1·31	7·2 ± 4·9	3·1 ± 2·2
Ugilec 141				
50 mg kg^{-1}	1·20 ± 0·08	1·94* ± 0·68	9·4 ± 2·2	1·5 ± 0·7
200 mg kg^{-1}	1·45 ± 0·18	1·34 ± 0·57	7·8 ± 1·7	1·7 ± 1·2
Aroclor 1254				
50 mg kg^{-1}	1·11 ± 0·11	1·15 ± 0·24	14·4 ± 11·0	3·3 ± 2·0
200 mg kg^{-1}	1·10 ± 0·08	2·61* ± 0·50	16·1* ± 4·1	10·7* ± 4·6
PCB-77				
50 mg kg^{-1}	1·10 ± 0·16	1·10 ± 0·41	22·2* ± 3·0	4·5 ± 1·9

* = significantly different from control ($p \leq 0·05$).
[a] = wet weight.
[b] = mg microsomal protein.

controls) and the Aroclor and PCB-77 groups (to 133 and 118%, respectively) (Table 1, Fig. 2). No significant increase in liver weight was observed in the Ah-nonresponsive DBA/2 strain (Table 1). There was no difference in body weight gain between controls and any of the dosed groups for both C57BL/6 and DBA/2 mice (data not shown).

Hepatic cytochrome P450 content and EROD and PROD activity

A significant increase of hepatic cytochrome P450 content was observed in C57BL/6 and DBA/2 mice following exposure to either Ugilec 141 or Aroclor 1254 (Table 1). In both mouse strains this increase was not significant for PCB-77. Ethoxyresorufin-*o*-deethylase (EROD) activity was greatly increased in the C57BL/6 mice to more than 3500% by PCB-77. This increase was much less in DBA/2 mice (308%). In C57BL/6 mice EROD

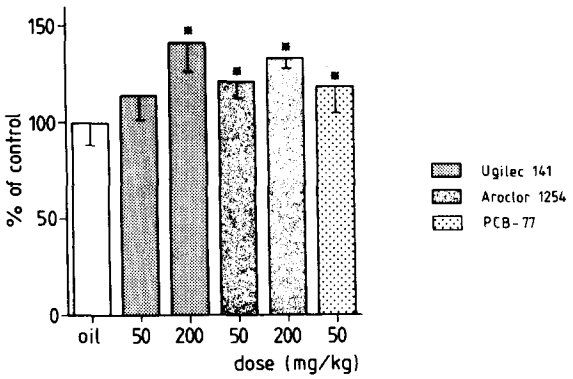

Fig. 2. Liver weight of C57BL/6 mice as a percentage of control * is significantly different from control.

activity was also increased by Ugilec 141 (252%) and by Aroclor 1254 (843%). In DBA/2 mice, EROD activity was also increased by Aroclor 1254 (224%). Pentoxyresorufin-*o*-deethylase (PROD) activity was significantly induced in both C57BL/6 and DBA/2 mice by Aroclor 1254 (respectively, 1680 and 345%), but not by Ugilec 141 (Table 1). PCB-77 significantly induced PROD only in C57BL/mice.

Plasma thyroxin and retinol level

The non-Ah mediated reductions in plasma total thyroxin (TT4) (Table 2) and plasma retinol (Table 3) concentrations were observed in both strains of mice, following exposure to either Ugilec 141, Aroclor 1254, or PCB-77 (Figs 3 and 4). Reductions of TT4 concentrations were already visible at 24 h

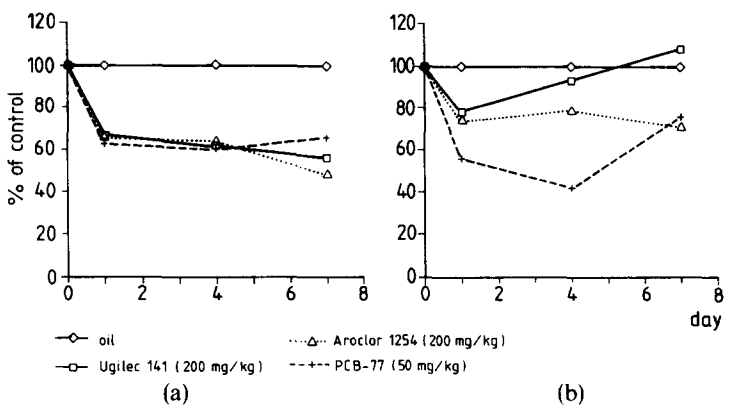

Fig. 3. Plasma Total Thyroxin (TT4) (a) and retinol (b) levels in C57BL/6 mice of the high dose groups, as a percentage of control (ranges are given in Table 2).

TABLE 2
Plasma Total Thyroid Hormone Levels (nmol litre^{-1}) at Different Times after Dosing, for the Different Dosages of Ugilec and PCBs

Mouse strain treatment	*Day 1*	*Day 4*	*Day 7*
C57BL/6			
Cornoil	16·9 ± 6·4	30·8 ± 6·3	34·1 ± 7·5
Ugilec 141			
50 mg kg^{-1}	19·3 ± 9·1	26·5 ± 6·0	23·4* ± 4·0
200 mg kg^{-1}	11·3 ± 2·8	19·1* ± 2·9	19·0* ± 1·8
Aroclor 1254			
50 mg kg^{-1}	17·1 ± 4·7	26·1 ± 4·8	23·3* ± 9·5
200 mg kg^{-1}	10·9 ± 3·1	19·6 ± 5·9	16·4* ± 4·6
PCB-77			
50 mg kg^{-1}	10·6 ± 2·6	18·4 ± 6·8	22·6* ± 4·9
DBA/2			
Cornoil	22·9 ± 4·6	38·5 ± 11·0	42·5 ± 4·1
Ugilec 141			
50 mg kg^{-1}	20·3 ± 4·9	35·1 ± 9·1	31·1 ± 8·4
200 mg kg^{-1}	16·6 ± 4·7	21·5 ± 6·2	26·3* ± 2·4
Aroclor 1254			
50 mg kg^{-1}	18·0 ± 3·8	29·7 ± 3·4	27·6 ± 5·5
200 mg kg^{-1}	22·6 ± 7·8	19·3 ± 4·1	25·7* ± 5·5
PCB-77			
50 mg kg^{-1}	18·9 ± 7·2	23·6 ± 7·8	18·9* ± 10·9

* = significantly different from control ($p \leq 0{\cdot}05$).

after exposure in both strains of mice. Figure 5 gives the plasma TT4 levels of C57BL/6 mice at day 7, and shows that the effects are clearly dose-related.

Plasma retinol reductions were at the lowest levels at day 4 in DBA/2 mice (for the high doses of Ugilec and Aroclor and for PCB-77, respectively, 56%, 41% and 33% of controls) and at day 1 in C57BL/6 mice (respectively, 78%, 74% and 56%).

Hepatic retinoid level

Hepatic retinoid (retinol and retinylpalmitate) concentrations were not altered in the DBA/2 mice. In the C57BL/6 mice, however, the hepatic retinol content was reduced by Ugilec 141, Aroclor 1254 and PCB-77 in the high dose groups to respectively, 66%, 43% and 85% of cornoil treated controls (Fig. 6). This reduction, however, was statistically significant only for both Aroclor 1254 groups.

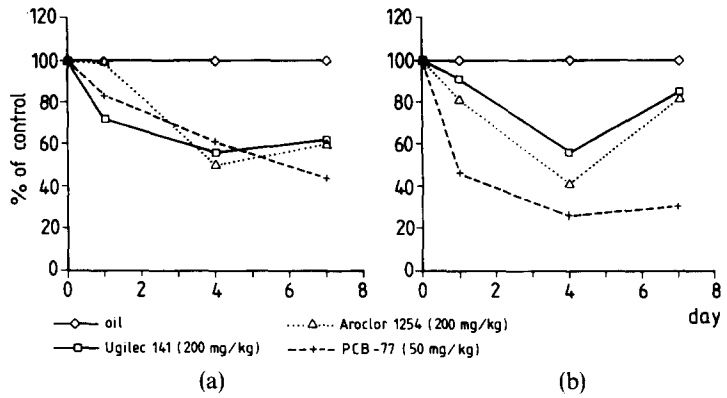

Fig. 4. Plasma Total Thyroxin (TT4) (a) and retinol (b) levels in DBA/2 mice of the high dose groups, as a percentage of control (ranges are given in Table 3).

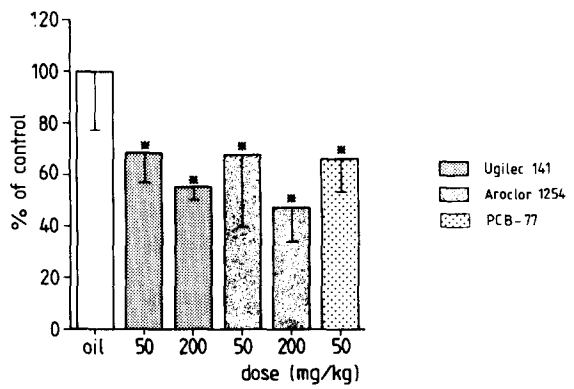

Fig. 5. Plasma Total Thyroxin (TT4) in C57BL/6 mice at day 7, as a percentage of control * is significantly different from control.

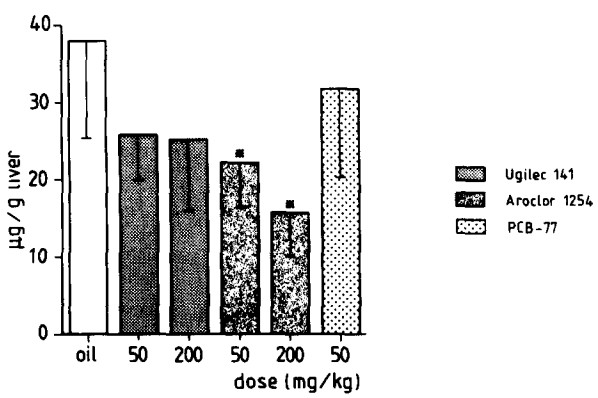

Fig. 6. Hepatic retinol levels in C57BL/6 mice * is significantly different from control.

TABLE 3
Plasma Retinol Levels (μg litre^{-1}) at Different Times after Dosing, for the Different Dosages of Ugilec and PCBs

Mouse strain treatment	*Day 1*	*Day 4*	*Day 7*
C57BL/6			
Cornoil	100 ± 16	109 ± 40	138 ± 30
Ugilec 141			
50 mg kg^{-1}	105 ± 20	103 ± 28	151 ± 65
200 mg kg^{-1}	78* ± 11	102 ± 22	149 ± 21
Aroclor 1254			
50 mg kg^{-1}	90 ± 23	46 ± 27	115 ± 32
200 mg kg^{-1}	74* ± 12	86 ± 27	100* ± 13
PCB-77			
50 mg kg^{-1}	56* ± 13	46* ± 12	105* ± 14
DBA/2			
Cornoil	62 ± 14	212 ± 6	154 ± 31
Ugilec 141			
50 mg kg^{-1}	59 ± 11	120* ± 39	133 ± 18
200 mg kg^{-1}	56 ± 8	119* ± 27	130 ± 12
Aroclor 1254			
50 mg kg^{-1}	54 ± 11	128* ± 29	130 ± 27
200 mg kg^{-1}	50 ± 15	86* ± 46	126 ± 36
PCB-77			
50 mg kg^{-1}	29* ± 5	70* ± 22	48* ± 10

* = significantly different from control ($p \leq 0{\cdot}05$).

Liver and thyroid pathology

In both C57BL/6 and DBA/2 mice histological alterations, such as mild centrilobular megalocytosis and occasionally necrosis, were observed especially in the Ugilec 141 (200 mg kg^{-1}) treatment group. Those symptoms were hardly visible in the PCB-77 groups. The thyroid gland showed mild hypertrophy of the thyroid follicle cells in both mouse strains, again especially following Ugilec 141 (200 mg kg^{-1}) treatment.

DISCUSSION

The technical TCBT-mixture Ugilec 141 causes a similar spectrum of toxicological alterations as the technical PCB-mixture Aroclor 1254 and the model toxic PCB-congener 3,3′,4,4′-tetrachlorobiphenyl (PCB-77). In addition, induction of hepatic cytochrome P450 and EROD activity and

reduction of levels of plasma retinol and thyroxin caused by Ugilec 141 were apparent in the same mouse strains as for Aroclor 1254 and PCB-77. This indicates a similar mechanism of action between the different compounds, although EROD activity was induced to a lesser extent by Ugilec 141 than by Aroclor 1254 and PCB-77. This may result from the fact that TCBTs deviate to a larger extent from a planar configuration than do the PCBs that have been tested. A planar configuration is an essential structural requirement for EROD induction (Goldstein & Safe, 1989).

PROD activity was not induced by Ugilec 141, but Aroclor 1254 induced PROD activity in both mouse strains. PROD activation is by isozymes that are not under control of the Ah-receptor. It is unknown whether intrinsic differences between TCBTs and biphenyls cause this difference or the fact that Ugilec 141 only consists of tetra-halogenated molecules, while Aroclor 1254 is a mixture of tri-, tetra-, penta-, and hexa-chlorinated biphenyls. The structural requirements for PROD induction are not yet elucidated.

The typical non-Ah mediated alterations in retinol and thyroid hormone levels were induced to the same extent for Ugilec 141, Aroclor 1254 and PCB-77 in both mouse-strains. This indicates that exposure of mice to Ugilec 141 leads to production of metabolites with a high binding affinity to transthyretin and accompanying distortion of plasma thyroxin and retinol transport (Brouwer & van den Berg, 1986). The distortion is qualitatively and quantitatively similar to PCB-77 and Aroclor 1254.

In view of its persistence, its bioaccumulation properties, the expected global dispersion and structural relationship with PCBs and DDT, Ugilec 141 can be expected to accumulate in adipose tissues and milk of animals and man. This property, combined with the fact that no apparent differences in toxicity were observed between Ugilec 141 and the PCBs, poses a risk for adverse effects in aquatic organisms and their predators, including human beings. In addition, Theisen *et al.* (1987) have shown that incomplete incineration of Ugilec 141 will lead to the formation and emission of polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs).

Ugilec 141 nevertheless was judged to be acceptable on the basis of data derived from standard toxicity screening tests, required for all new substances before entering the European market. However, in the early 1970s it was already known that PCBs showed a relatively low acute toxicity in standard screening assays, but induced a severe and complex pattern of lesions in various animals and man. In other words, the PCB problem showed that a standard screening test-protocol is not valid for this class of chemicals.

Instead, we would recommend that more attention should be paid to the capacity for bioaccumulation of a substance. Expected high levels of

accumulation should be important enough to prevent registration of a substance. When bioaccumulation capacity is not high enough to prevent registration, it is important that in further toxicity testing procedures more specific toxicity parameters are included, based on the mechanism of action of the chemical(s) under investigation. Studying mechanisms of action also provides information about possible additivity, for Ugilec with substances like PCBs, PCDDs, etc. In the case of Ugilec or other PCB substitutes we would advise to use the parameters investigated in this study.

Hopefully the case of Ugilec 141 will help to prevent the introduction of related compounds such as dibromobenzyltoluenes.

CONCLUSION

No apparent differences were observed in toxicity between Ugilec 141 and the PCBs, Aroclor 1254 and PCB-77. This turns Ugilec 141 and related TCBTs into very bad alternatives for PCBs.

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REFERENCES

Brouwer, A. (1989). Inhibition of thyroid hormone transport in plasma of rats by polychlorinated biphenyls. *Arch. Toxicol., Suppl.* **13**, 440–5.

Brouwer, A. & van den Berg, K. J. (1986). Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. *Toxicol. Appl. Pharmacol.*, **85**, 301–12.

Friege, H., Stock, W., Alberti, J., Poppe, A., Juhnke, I., Knie, J. & Schiller, W. (1989). Environmental behaviour of polychlorinated monomethyl-substituted diphenyl-methanes (Me–PCDMs) in comparison with polychlorinated biphenyls (PCBs). II: Environmental residues and aquatic toxicity. *Chemosphere*, **18**, 1367–78.

Fürst, P., Krüger, C., Meemken, H-A. & Groebel, W. (1987). Determination of the polychlorinated biphenyl substitute Ugilec (tetrachlorobenzyltoluenes) in fish. *J. Chrom.*, **405**, 311–7.

Goldstein, J. A. & Safe, S. (1989). Mechanism of action and structure-activity relationship for the chlorinated dibenzo-*p*-dioxins and related compounds. In *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzo-*p*-dioxins and Related Products.* ed. R. D. Kimbrough & A. A. Jensen, Elsevier Science Publishers, Inc., New York, pp. 239–93.

Lubet, R. A., Mayer, R. T., Cameron, J. W., Nims, R. W., Burke, M. D., Wolff, T., Guengerich, F. P. (1985). Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Archives of biochemistry and biophysics*, **238**(1), 43–48.

Omura, T. & Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.*, **239**, 2370–85.

Peter, H., Karl, W., Roll, G. & Schlatterer, B. (1989). Ersatzstoffe für Kondensatoren, Transformatoren und als Hydraulikflüssigkeiten im Untertagebergbau verwendete Polychlorierte Biphenyle. Umweltbundesamt, Berlin, 96 pp.

Poppe, A., Alberti, J. & Friege, H. (1988). Ökotoxikologische Einschatzung des PCB-Ersatzstoffes Ugilec 141. *GewässerschutzWasser-Abwasser*, **105**, 58–88.

Prough, R. A., Burke, M. D. & Mayer, R. T. (1978). Direct fluorimetric methods for measuring mixed function oxidase activity. In *Methods in Enzymology*. ed. S. Fleisher & L. Packer, Vol. 52. Academic Press, New York, pp. 372–7.

Theisen, J., Funcke, W., Balfanz, E., König, J., Poppe, A. & Friege, H. (1987). Entstehung von polychlorierten Dibenzofuranen (PCDFs) und Dibenzo(*p*)-dioxinen (PCDDs) durch Pyrolyse von Ugilec 41 (Posterbeitrag). *VDI Berichte*, **634**, 265–76.

Wester, P. G. & van der Valk, F. (1990). Tetrachlorobenzyltoluenes in Eel from the Netherlands. *Bull. Environ. Contam. Toxicol.*, **45**, 69–73.