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The Relevance of Fat Content in Toxicity of Lipophilic Chemicals to Terrestrial Animals with Special Reference to Dieldrin and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

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Lipophilic chemicals such as chlorinated hydrocarbon insecticides and other *persistent* chemicals such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are fat soluble chemicals and are readily bioconcentrated in animal fat depots. The modifying role of the body fat content in the toxicity of chlorinated cyclodiene insecticides to insects and in the toxicity of TCDD to different mammals was investigated. The single oral acute 30-day LD_{50} data of TCDD in different mammals are presented and correlated with their total body fat content. A two linear regression equation with log/log values was obtained. It is concluded that the storage of TCDD and other related lipophilic and persistent chemicals in lipids of organisms is, in a sense, a detoxication mechanism by which the compounds are removed from sites of action and/or receptors. Therefore, terrestrial organisms such as insects and mammals with higher total body fat content can accumulate and tolerate higher chlorinated hydrocarbon insecticide and TCDD doses than organisms with lower fat content. The different sensitivity of mammals of various species, strains, body weight, sex, age, etc. to acute toxicity of TCDD and related lipophilic persistent chemicals can mainly be explained by differences in total body fat content.

1. INTRODUCTION

The acute toxicity (LD_{50}) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was determined for many different animal species. Young male guinea pigs were by far the most sensitive species tested: their single oral 30-day LD_{50} value was between 0.6 and 2.5 μg/kg body weight. Adult Syrian hamsters were the least sensitive mammals tested thus far. Their single oral 30-day LD_{50} value is between 2530 and 8420 times as great as that of the young guinea pigs (Schwetz *et al.*, 1973; McConnell *et al.*, 1984; Olson *et al.*, 1980; Henck *et al.*, 1981).

This marked species difference in TCDD toxicity has been an unresolved problem for more than a decade. Elucidating the interspecies differences in TCDD toxicity and the mechanism of action of TCDD has been the subject of intense study during the past decade. But in our view none of the hitherto advanced hypotheses are capable of providing a satisfactory explanation for the marked species differences in TCDD toxicity.

After intensive studies of literature, the authors came to the conclusion that the modifying role of the total body fat content of the organisms has largely been forgotten when the toxic effects of halogenated aryl hydrocarbons and other lipophilic chemicals

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including TCDD have been estimated. It was considered worthwhile to investigate if there exist relationships between toxicity of lipophilic chemicals in different organisms and their lipid content. The main part of this paper deals with the acute toxicity (LD_{50}) of chlorinated hydrocarbon insecticides to different insects and of lipophilic persistent chemicals, especially TCDD, in different mammals and their total body lipid content.

2. TOXICITY OF LIPOPHILIC CHEMICALS TO INSECTS AND THEIR LIPID CONTENT

2.1. Previous Studies

Munson and Gottlieb (1953) proposed first the increase of lipid content as a protective mechanism against DDT toxicity in the American cockroach, *Periplaneta americana* (L.). In one *Anopheles* strain (*A. atroparvus* van Thiel), a dieldrin-selected strain contained significantly more total lipid than the original susceptible strain (Neri *et al.*, 1962). The increase in tolerance to heptachlor of alfalfa weevils, *Hypera postica*, as they became older, was correlated with the increase in their total fat content by Bennett and Thomas (1963).

Moriarty (1968) studied the toxicity of dieldrin and DDT to insects and found that the LD_{50} values were increasing, i.e., the toxicity was decreasing with the total lipid content of the insects.

Reiser *et al.* (1953) found that adult boll weevils (*Anthonomus grandis Boheman*) that survived given applications of dieldrin or toxaphen were found to contain at least twice as much total lipid as the adults which succumbed. It was suggested that the higher fat content, larger size, and increasing resistance of late season weevils may be due to the nutritional advantage of boll reared over square reared insects.

2.2. Toxicity of Dieldrin to Insects and Correlation with Their Lipid Content

Khan and Brown (1966) investigated the acute toxicity (LD_{50}) of dieldrin against different strains of larvae of the yellow-fever mosquito, *Aedes aegypti* (L.), by the standard WHO method for mosquito larvae. They also determined their total lipid content by extraction with a 2:1 mixture of chloroform–methanol (Folch *et al.*, 1957).

The data of their investigations are presented in Table 1. The log LC_{50} values of the different strains was correlated with their total fat content (TBF) in percentages on a wet weight basis. The following regression Equation (1) was obtained:

$$\log LC_{50} = 1.83 \cdot \mathrm{TBF} - 8.18. \qquad (1)$$

The correlation coefficient is $r = 0.855$, the standard error of the estimate (square root of the variance of estimate) $S_{y.x} = 0.789$, and the number of data points used in the calculation of the regression equation was $N = 12$. The positive correlation is highly significant ($r = 0.855$; significance level: $P < 0.001$). The graphic expression of Equation (1) is presented in Fig. 1.

3. TOXICITY OF LIPOPHILIC CHEMICALS TO MAMMALS AND THEIR FAT CONTENT

3.1. Previous Studies

During toxicological observations, Spicer *et al.* (1947) found that goats in good nutritional state with average amount of body fat tolerated a much larger dose of

TABLE 1

ACUTE TOXICITY (LC_{50}) AND TOTAL LIPID CONTENT OF DIFFERENT STRAINS OF MOSQUITO LARVAE (*Aedes aegypti*) AGAINST DIELDRIN

Strain or generation	LC_{50} (ppm)	log LC_{50}	Lipid ± SD[a] (%)
Karachi (N)[b]	0.01	−2.0	3.09 ± 0.11
Karachi (R)[c]	29.00	1.46	4.57 ± 0.12
Kongolikan (N)	0.007	−2.15	3.77 ± 0.07
Kongolikan (R)	4.60	0.66	4.60 ± 0.03
Trinidad S	0.016	−1.80	4.23 ± 0.10
Isla Verde	0.31	−0.51	4.39 ± 0.24
Hybrids[d]	0.79	−0.10	4.52 ± 0.32
Isla Verde (N)	1.10	0.04	4.63 ± 0.3
Isla Verde F_7-rel.	8.00	0.90	4.75 ± 0.25
Isla Verde F_3	20.00	1.30	4.97 ± 0.07
Isla Verde F_5	41.00	1.61	4.98 ± 0.018
Isla Verde F_7 (R)	70.00[e]	1.85	5.88 ± 0.11

Note. Reference: Khan and Brown (1966).
[a] Total lipid content on a wet weight basis ± standard deviation (SD).
[b] N, normal.
[c] R, resistant.
[d] Hybrids: Trinidad S × Isla Verde F_5.
[e] Calculated by the authors.

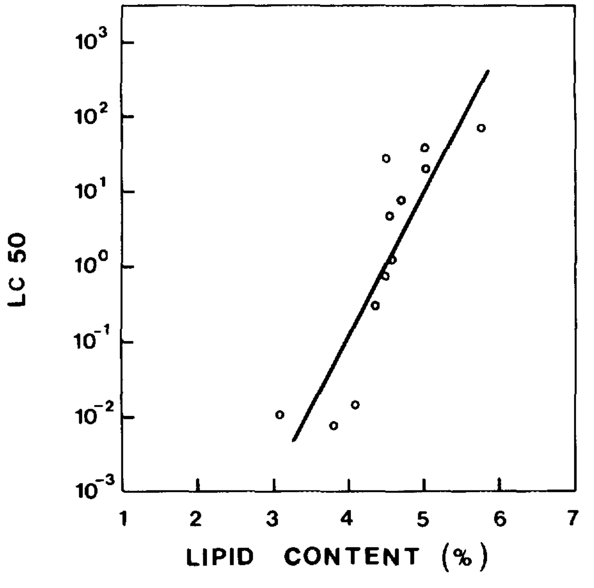

FIG. 1. Relationship between median lethal concentration (log LC_{50}) of dieldrin and different strains of mosquito larvae and their total fat content (%).

DDT than those in poor nutritional state with lower total body fat content. Gak *et al.* (1976) compared the lethal doses of *p,p'*-DDT, DDE, chlordane, heptachlor, aldrin, dieldrin, lindane, and endosulfan in mice, rats, and golden hamsters. In all these cases the LD_{50} data of hamsters were greater than those of mice and rats. The LD_{50} of *p,p'*-DDT in hamsters was more than 50 times greater in contrast to the LD_{50} of mice and rats. Gak *et al.* (1976) found also that the hamster was insensitive to *p,p'*-DDT and its metabolites DDE and DDD, so that an LD_{50} value could not be established.

Matthews and Anderson (1975) experimented on the role of adipose tissue in protection against polychlorinated biphenyls (PCBs) toxicity in rats. Decad *et al.* (1981) reported the toxic effects of 2,3,7,8-tetrachlorodibenzofuran (TCDF) in two mice strains and pointed out that the higher adipose tissue content of DBA/2J mice compared to C57BL/6J mice might serve as a protective reservoir for toxic effects of TCDF.

Recently, Ahotupa and Mäntylä (1983) investigated the role of adipose tissue content as a modifier of biological effects, tissue distribution, and excretion of 2,4,5,2',4',5'-hexachlorobiphenyl in two strains of mice.

3.2. Toxicity of TCDD to Mammals and Correlation with Their Fat Content

The examples presented above show that total body fat content influences the toxicity of lipophilic chemicals to higher organisms, in a similar manner as it influences bioconcentration (Geyer *et al.*, 1985). One of the main toxic effects of TCDD in all tested mammals is a loss of body weight, the so-called *wasting syndrome*. The loss is mainly contributed to body fat of the animals. Therefore, it seems logical also to look if there exists a relationship between the toxic potential of TCDD in different animals and their total body fat content. Of all the toxicological data, lethal toxicity—the dose of TCDD which, when administered by a certain route and schedule, kills a selected percentage (50%, i.e., the LD_{50}) during a specified observation period (30 days in this case)—is the easiest to measure with reasonable precision (OECD, 1987). Therefore, since there are many data on acute oral toxicity of TCDD in various laboratory animals in literature, the 30-day LD_{50} values were used for the quantitative correlation calculations.

3.2.1. Acute toxicity data of TCDD. The acute oral toxicity data (LD_{50}: median lethal dose) of TCDD in different species of laboratory mammals were taken from original literature and are compiled with the references in Table 2. The LD_{50} data are influenced by many factors such as animal species, strain, body weight, age, sex, health, diet, food deprivation, termination of time, etc. The method of TCDD administration (oral, intraperitoneal, dermal, subcutan, etc.) is also of great influence on the LD_{50} value (Olson *et al.*, 1980). In Table 2, therefore, these main informations are also given and only single *oral* lethal dose data which kill 50% of the animals in the mean time of 30 days are presented. The route of TCDD administration in oil by gavage was also nearly equal. Only in some cases oil and acetone was used as a vehicle (Table 2). In case that important data such as body weight, age, sex, vehicle, season of performing the toxicity test, etc. were not reported in the original publications, these data were received directly from the authors.

3.2.2. Total body fat content data. The total body fat content of some animals of the same strain, body weight, and/or age for which the LD_{50} values of TCDD are given in literature was determined according to the following procedure. Three animals were weighed individually, sacrificed, and dried to a constant weight in an oven at

110°C (time: mice for 2 days, hamsters for 3 days, and rats for 4 days, respectively). The dried carcass was ground in a mortar, mixed with perchloroethylene, and stored for 24 hr at room temperature. The total fat content was then extracted by the FOSS–LET method according to the operating instructions of the apparatus manufacturer Foss Electric (Skandinavien), A/S, 1 Huginsvej, DK-3400 Hilleroad, Denmark. The authors used their own calibration curve (specific gravity as a function of the fat content) and calculated the percentage total body fat content on a wet weight basis.

The other total body fat contents (percentage) of the remaining animals of the same strain, of approximately the same body weight and/or age were obtained from the literature. The fat content of all of these animals—except for the Rhesus monkeys—was determined physicochemically by extraction with organic solvents (petrolether, diethylether, etc.). In the case of the monkeys the percentage body fat was estimated by Bowman (1988), via the tritiated water method (Walker *et al.*, 1984). These data and data herein are compiled with references in Table 3.

3.2.3. Correlation of LD_{50} data of TCDD in mammals with their total fat content. The used FOSS–LET method for fat determination is in good agreement (Rapp, 1987; Herberg, 1987; Domanski *et al.*, 1974) with the other extraction methods taken from the literature. Therefore, the authors could use these data also for the correlation with the acute oral toxicity of TCDD in mammals.

A direct comparison of the acute oral toxicity of TCDD in different species and strains of animals is not hampered, and it was possible to investigate if there existed a relationship between the LD_{50} values of the different animals and their lipid content. For the correlation LD_{50} data were used for which the mean of time from dosing with until death was nearly equal (30 days).

The single oral median lethal doses (30-day LD_{50}) of TCDD in different animals were correlated with their total body fat content (percentage on a fresh weight basis).

Using a two variable linear regression with log values, the following regression Equation (2) was obtained:

$$\log LD_{50} = 5.30 \cdot \log(\mathrm{TBF}) - 3.22. \qquad (2)$$

LD_{50} is a statistically derived single median oral dose of TCDD in micrograms per kilogram body weight which kills 50% of the animals in an average time of 30 days. TBF is the total body fat content in percentage of body wet weight of the tested animals. The correlation coefficient is $r = 0.913$, the significance level $P < 0.001$, the standard error of the estimate $S_{y,x} = 0.460$, and the number of data points used in the calculation of the regression equation was $N = 20$.

The graphic expression of Equation (2) is presented in Fig. 2.

4. DISCUSSION

4.1. Toxicity of Chlorinated Hydrocarbons to Insects and Their Lipid Content

A statistical investigation revealed a close positive correlation ($r = 0.87$) between the acute toxicity (LC_{50} values) of the highly lipophilic chlorinated hydrocarbon dieldrin (*n*-octanol/water partition coefficient; $\log K_{ow}$: 4.32) to different strains and generations of the yellow-fever mosquito larva (*Aedes aegypti*) and their total lipid content. That means that the resistance of the mosquito is increasing with their increase in total lipid content. In the literature, other examples are found, where the increase in lipid

TABLE 2

SINGLE ORAL 30-DAY LD_{50} VALUES OF 2,3,7,8-TCDD IN DIFFERENT SPECIES AND STRAINS OF MAMMALS

No.	Species (strain)	Sex	Initial body weight gram[h] (age)	Route of administration (vehicle)	Time to death (days)[h]	LD_{50}[f] (μg/kg body wt.)	Reference
1a	Guinea pigs (Pirbright-white, Dunkin-Hartley)	M	200 (ca. 3 weeks)	Gavage (corn oil/benzene, 346:1)	21	0.7[c] (0.6–0.8)[b]	Poiger *et al.*, 1982
1b	Guinea pigs (Hartley)	M	359 ± 30	Gavage (corn oil/acetone, 9:1)	24.5 (19–30) 0.5 μg/kg group	0.6 (0.4–0.9)[a]	Schwetz *et al.*, 1973; Gehring and Betso, 1988
1c	Guinea pigs (Hartley)	M	423 ± 11		37 (32–42) 2.0 μg/kg group	2.1 (1.5–3.0)[a]	Schwetz *et al.*, 1973; Gehring and Betso, 1988
1d	Guinea pigs (Hartley)	M	213.5 ± 8.2 173–238 (2.5 weeks)		5–21	1.75 (1.26–2.24)[a]	McConnell *et al.*, 1984; McConnell, 1987
1e	Guinea pigs (Hartley)	M	200–250 (3–4 weeks)	Gavage (corn oil)	17–20 (median)	2.0	McConnell *et al.*, 1978b
1f	Guinea pigs (Hartley)	F	345–510 (7–10 weeks)		32–42 (2.5 μg/kg group)	2.5[e] (1.2–5.4)[a]	Silkworth *et al.*, 1982
2	American dark mink *Mustela vison*	M	1513 (1256–1809) ca. 2 years	Gavage (corn oil/acetone, 4:1)	28	4.2[i]	Hochstein *et al.*, 1988
3a	Rabbits harecolored	M	ca. 3000	Gavage (olive oil)		10	Schulz, 1968; Schulz, 1987
3b	Rabbits (New Zealand White)	M + F	2000–3000	Gavage (corn oil/acetone, 9:1)	6–39	115 (38–345)[a]	Schwetz *et al.*, 1973; Gehring and Betso, 1988
4	Rhesus monkeys	F	2100–2600	Gavage (corn oil)	14–47	<70	McConnell *et al.*

	Macaca mulatta		(juvenile)			50	
5a	Rats (Sherman, Spartan)	M	251 ± 32 15.8 + 31.6 μg/kg group (202–309)	Gavage	26 31.6 μg/kg group (19–43)	22 (15.8–31.6)[b]	Sc
5b	Rats (Sherman, Spartan)	F	174 ± 14 31.6 + 63 μg/kg group (156–192)	(corn oil/acteone, 9:1)	28 31.6 + 63 μg/kg group (18–43)	45 (30–66)[a]	Sc
5c	Rats (Fischer F 344)	F	100–130 (ca. 4–5 weeks)	Gavage (corn oil)	N.R.	40	Al
5d	Rats (Fischer F 344)	M	130–150	Gavage (corn oil)	14	47	M
5e	Rats (Sprague-Dawley SAS: VAF/1 SD)	M	290.2 ± 2.6 (ca. 8.6–10 weeks)	Gavage (corn oil/acetone 19/1)	31	43	St
5f	Rats (Sprague-Dawley)	M	200 (6–7 weeks)	Gavage (corn oil)	25	50	Al
5g	Rats (Gun, heterozygous, GW)	F	N.R.	N.R.	28.5 ± 7.4 (18–84)	60	Th
5h	Rats (Gun, heterozygous, GW)	M	N.R.	N.R.	28.5 ± 7.4 (18–48)	93	Th
5i	Rats (Gun, homozygous, GG)	F	N.R.	N.R.	28.5 ± 7.4 (18–48)	116	Th
5j	Rats (Gun, homozygous, GG)	M	N.R.	N.R.	28.5 ± 7.4 (18–48)	280	Th
5k	Rats, outbred (CD, Sprague–Dawley)	F	168.8 ± 4.9 (6–8 weeks)	Gavage (corn oil/acetone, 6.4:1)	18–21	100	Ha
5*l*	Rats, outbred (Charles River, CR/CD, Sprague–Dawley)	M	352 ± 5 (10–11 weeks)		24.5 ± 1.0	297 (240–360)[a]	W
5m	Rats (Charles River, Fischer, CR/F 344 N)	M	265 ± 2 (11–12 weeks)	Gavage (corn oil)	24.8 ± 0.6	164 (104–217)[a]	W

TABLE 2—*Continued*

No.	Species (strain)	Sex	Initial body weight gram[h] (age)	Route of administration (vehicle)	Time to death (days)[h]	LD_{50}[f] (μg/kg body wt.)	Reference
5n	Rats (Frederick, Fischer, F/F 344N)	M	240 ± 4 (11–12 weeks)	Gavage (corn oil)	25.9 ± 0.8	303 (250–360)[a]	Walden and Schiller, 1985
5o	Rats (Harlan, Fischer, H/F 344N)	M	268 ± 5 (11–12 weeks)		28.3 ± 0.5	340 (281–409)[a]	Walden and Schiller, 1985
5p	Rats (Hannover, Wistar), outbred	M	282 ± 4 (12 weeks)	Gavage (corn oil)	64[j]	>1000	Pohjanvirta *et al.*, 1987; Pohjanvirta, 1988
6a	Mice (C57BL/6Sch)	M	27.1 23.6–30.8 (12 weeks)	Gavage (corn oil/acetone, 9:1)	15–20 (200 μg/kg group)	114	Vos *et al.*, 1974
6b	Mice (C57BL/6)	M	14–30 (7–15 weeks)	Esophageal	21 ± 1.6	126 (86–183)[a]	Jones and Greig, 1975
6c	Mice (C57BL/10)	M	N.R. (6–17.3 weeks)	Intubation (arachis oil)	22–26 (most death)	146 (111–211)[a]	Smith *et al.*, 1981
6d	Mice (C57BL/6J)	M	25.8–26.3 (10–12 weeks)		24.4 ± 0.7	182 (163–201)[a]	Chapman and Schiller, 1985
6e	Mice (C57BL/6fh)	M	21–25 (9 weeks)	Gavage	22–25 (median)	283.7	McConnell *et al.*, 1978b
6f	Mice (B6 $D2F_1$/J)[d]	M	22–32 (10–12 weeks)	(corn oil)	25.4 ± 0.6	296 (268–324)[a]	Chapman and Schiller, 1985
6g	Mice (BALb/c)	M	18–26 (8.6–9.9 weeks)	Gavage (corn oil)	28.9 (23–37)	399[g]	Greig, 1987
6h	Mice (C57BL/10)	F	N.R. (6–17.3 weeks)	Esophageal intubation (arachis oil)	N.R.	>450	Smith *et al.*, 1981
6i	Mice (DBA/2J)	M	27.6–28.9 (10–12 weeks)	Gavage (corn oil)	21.4 ± 0.7	2570 (2206–2912)[a]	Chapman and Schiller, 1985
6j	Mice (C57 BL/6J-$Ah^{b/b}$)	M	29.2 ± 0.7 (12 weeks)	Gavage (corn oil)	22	159 (125–193)[a]	Birnbaum *et al.*, 1990
6k	Mice (C57 BL/6J-$Ah^{d/d}$)	M	28.7 ± 0.8 (12 weeks)	Gavage (corn oil)	22	3,350 (2809–4360)[a]	Birnbaum *et al.*, 1990

7a	Dogs (Beagle)	M	ca. 7000–13,000	Gavage (corn oil/acetone, 9:1)	9–15	1000[c] (300–3000)[b]	Schwetz *et al.*, 1973; Gehring and Betso, 1988
7b	Dogs (Beagle)	F	13,100 ± 4100 (8000–17,500)	Gavage (corn oil/acetone, 9:1)	No death	>100	Schwetz *et al.*, 1973; Gehring and Betso, 1988
8a	Hamsters (golden Syrian) *Mesocricetus auratus*	M	49.3 44–54 (ca. 3–4 weeks)	Gavage (corn oil)	32 (15–47)	1157 (829–1583)[a]	Olson *et al.*, 1980; Olson, 1988
8b	Hamsters, outbred (golden Syrian) *Mesocricetus auratus*	M	101.8 ± 10.3[k] 70–120 (ca. 8 weeks)	Gavage (corn oil/acetone, 9:1)	30 (9–43)	5051 (3876–18,487)[a]	Henck *et al.*, 1981; Betso, 1988; Rao, 1988

Note. N.R., not reported.
[a] 95% confidence intervals.
[b] The number in parentheses indicate the range of lethal doses (0 and 100% mortality).
[c] Calculated by the authors.
[d] (C57BL/6J female × DBA/2J male) F_1.
[e] 42-day LD_{50}.
[f] Amount (μg/kg body wt.) required to kill 50% of the animals by 30 days (unless otherwise indicated) postexposure.
[g] 45-day LD_{50}.
[h] Mean ± SE and/or range.
[i] 28-day LD_{50}. Minks were dosed in June.
[j] Duration of observation.
[k] Mean initial body weight of subset II hamsters which were dosed 1000, 3000, and 6000 μg/kg (see Henck *et al.*, 1981).

TABLE 3

SINGLE ORAL 30-DAY LD_{50} VALUES OF 2,3,7,8-TCDD IN DIFFERENT MAMMALS AND THE TOTAL BODY FAT CONTENT (TBF) OF ANIMALS COMPARABLE WITH THOSE FROM TABLE 1 (For Further Information See Table 1: Numbers Refer to Table 1)

No.	Species (strain)	LD_{50}[f] (μg/kg body wt.)	References	Sex	Body weight[h] (g)	Total body fat content[h] (% body wt.)	References
1a	Guinea pigs (Pirbright-white, Dunkin-Hartley)	0.7[c] (0.6–0.8)[b]	Poiger *et al.*, 1982	M	195.8 ± 3.5	4.5 ± 0.84	This work
1d	Guinea pigs (Hartley)	1.75 (1.26–2.24)[a]	McConnell *et al.*, 1984; McConnell, 1987	M	195.8 ± 3.5[j]	4.5 ± 0.84[j]	This work
1e	Guinea pigs	2.0	McConnell *et al.*, 1987b	M	195.8 ± 3.5[j]	4.5 ± 0.84[j]	This work
3a	Rabbits	10	Schulz, 1968; Schulz, 1987	M	2560	7.5	Spray and Widdowson, 1950
3b	Rabbits (New Zealand White)	115	Schwetz *et al.*, 1973; Gehring and Betso, 1988	M + F	2580	10.1	Spray and Widdowson, 1950
4	Rhesus monkeys *Macaca mulatta*	50	McConnell *et al.*, 1978a; McConnell, 1987	F	2340	10.3	Bowman, 1988
5c	Rats (Fischer F 344)	40	Albro *et al.*, 1978	F	113.3 ± 2.2	10.8 ± 2.3	This work
5d	Rats (Fischer F 344)	47	McConnell *et al.*, 1978a; McConnell, 1987	M	143.5 ± 5.3	10.1 ± 1.4	This work
5e	Rats (Sprague–Dawley)	50	Allen *et al.*, 1975	M	187.7 ± 5.3	8.9 ± 0.14	This work
5j	Rats, outbred (CD, Sprague–Dawley)	100	Harris *et al.*, 1973	F	166.1 ± 2.8	11.4 ± 0.45	This work
5k	Rats, outbred (CR/CD, Sprague–Dawley)	297 (240–360)[a]	Walden and Schiller, 1985	M	344.5 ± 3.6	9.4 ± 0.3	This work

5m	Rats (Frederick, Fischer, F/F 344N)	303 (250–360)[a]	Walden and Schiller, 1985	M	250.0 ± 7.1	10.5 ± 0.2	This v
6d	Mice (C57 BL/6J)	182 (163–201)[a]	Chapman and Schiller, 1985	M	25.1 ± 1.4	7.9 ± 1.5	Ahotu 198
6e	Mice (C57 BL/6fh)	283.7	McConnell *et al.*, 1978b	M	23.6 ± 2.7	9.8 ± 0.2	This v
6f	Mice (B6 $D2F_1$/J)	296 (268–324)[a]	Chapman and Schiller, 1985	M	27.7 ± 2.8	14.3 ± 1.9	This v
6i	Mice (DBA/2J)	2570 (2206–2912)[a]	Chapman and Schiller, 1985	M	27.23 ± 0.4	20.0 ± 2.6	This v
7a	Dogs (Beagle)	1000[c] (300–3000)[b]	Schwetz *et al.*, 1973; Gehring and Betso, 1988	M	9,220 ± 2,190 (7,180–13,070)	13.8 ± 4.8	Sheng 197
8a	Hamsters (golden Syrian) *Mesocricetus auratus*	1157 (829–1583)[a]	Olson *et al.*, 1980; Olson, 1988	M	46.8 ± 0.3	13.7 ± 0.9	This v
8b	Hamsters, outbred (golden Syrian)	5051[k] (3,876–18,487)[a]	Henck *et al.*, 1981; Betso, 1988; Rao, 1988	M	96.2 ± 0.35	17.3 ± 0.4	This v
	Mesocricetus auratus	5051[k]	Henck *et al.*, 1981	M	127.4 ± 6.4	20.2 ± 4.3	Koda

Note. N.R., not reported.

[a] 95% confidence intervals.

[b] The number in parentheses indicate the range of lethal doses (0 and 100% mortality).

[c] Calculated by the authors.

[d] (C57 BL/6J female × DBA/2J male) F_1.

[e] 42-day LD_{50}.

[f] Amount (μg/kg body wt.) required to kill 50% of the animals by 30 days (unless otherwise indicated) postexposure.

[g] 45-day LD_{50}.

[h] Mean ± SE and/or range.

[i] 28-day LD_{50}.

[j] Pirbright-white, Dunkin-Hartley strain.

[k] Subset I hamsters were dosed on September 20, 1979 and subset II hamsters on January 8, 1980.

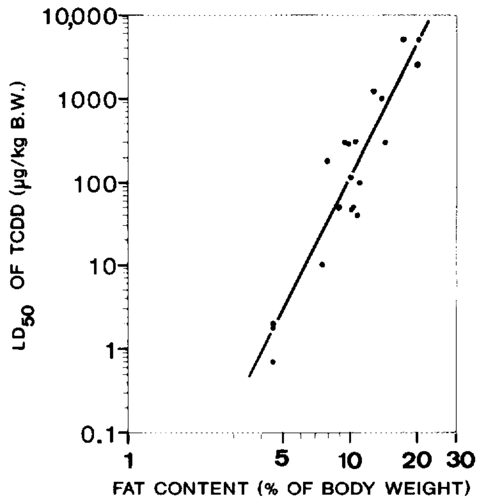

FIG. 2. Relationship between the medium lethal oral doses (LD_{50}) of TCDD in different mammals and their body fat content (% body wt.) (log–log scale).

content of insects reacts as a protective mechanism against lipophilic cyclodiene insecticides and chlorinated hydrocarbons, as discussed above (Neri *et al.*, 1962; Reiser *et al.*, 1953).

However, it must be pointed out that the resistance to toxicity of lipophilic chlorinated hydrocarbons used as insecticides is not universally related to the total lipid content in insects. A highly dieldrin-resistant strain of house flies contained no more lipid than a susceptible strain (Bridges and Cox, 1959). Bradbury *et al.* (1958) found that strains of *Anopheles gambiae* and house flies resistant to dieldrin and lindane contained smaller or equal amounts of lipids than the susceptible strains.

For a long time it has been known that pesticide resistance is frequently due to different mechanisms in different strains of insects. In addition to quantitative or qualitative differences of tissue lipid or fat content in insects, other mechanisms could be responsible for the development of resistance to a once-selected insecticide or other insecticides, such as differences in absorption, metabolism, excretion, hydroxylation steps involved in growth and/or moulting hormone systems, electron transporting systems, variety of metabolic detoxication reactions such as dehydrochlorination or conjugation process which often require a single cofactor such as gluthathion or reduced nicotinamide adenine dinucleotide phosphate (NADPH), etc. The resistance might be explained by the presence of a gene or gene groups which is/are responsible for the control of these biochemical mechanisms (Metcalf, 1989). In the case of DDT resistance of house flies it was found that these resistant insects are able to dechlorinate DDT to nontoxic DDE at a faster rate than susceptible house flies.

Nevertheless, the above-presented example has clearly shown that the total lipid content of insects could play a main role in resistance of insects to the cyclodiene insecticide dieldrin.

TABLE 4

FACTORS WHICH CAN INFLUENCE TOXICITY OF TCDD AND RELATED LIPOPHILIC CHEMICALS IN TERRESTRIAL ORGANISMS

Internal factors		External factors
1. Species 2. Strain 3. Genetic background (a) Inbred (b) Outbred (c) Homocygous (d) Heterocygous 4. Sex (a) Male (b) Female (c) Castrated 5. Body weight 6. Age (young or adult) 7. Health status 8. Females used or not used for breeding 9. Hormone status	Total Body Fat Content	1. Diet (high or low energy) 2. Season of the year 3. Environment factors (a) Temperature (b) Humidity (c) Light–dark cycle, etc. 4. Stress 5. Food deprivation 6. Time of dosing 7. Duration of dosing 8. Formulation of the chemical 9. Vehicle, volume 10. Housing conditions (design and size of cage, type of litter, number of animals kept in one cage) 11. Route of administration (oral, dermal, intraperitoneal, intravenous, inhalation, etc.)

4.2. *Toxicity of TCDD to Mammals and Their Lipid Content*

The above investigations indicate an obvious linear-positive relationship between the logarithm of the single medium lethal oral dose (log LD_{50}) of TCDD and the logarithm of the total body fat content (log TBF%) of the tested animals. The positive correlation is significant ($r = 0.913$; significance level: $P < 0.001$; $N = 20$).

5. CONCLUSION

The authors came to the conclusion that there seems to be a common mechanism for the toxic action of TCDD which reacts like an "*artificial wasting hormone*." The different sensitivity of mammals of various species, strain, body weight, sex, age, etc. to toxicity of TCDD and other lipophilic persistent compounds could mainly be explained by differences in the *total body fat* (brown adipose tissue plus white adipose tissue) content. In this adipose tissue, lipophilic persistent chemicals are mainly bioconcentrated so that only a relatively small fraction of the administered dose can reach target organs and/or receptors to execute its toxic effects. Therefore, it is concluded that the adipose tissue serves as a protective reservoir against the toxic effects of TCDD and other lipophilic persistent chlorinated compounds such as DDT, dieldrin, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Therefore, animals with low total body lipid content such as *young* guinea pigs, rabbits, and other *young* herbivores are more sensitive to toxic effects of TCDD and related chemicals than animals with higher lipid content such as *adult* hamsters and other *adult* omnivores.

6. RECOMMENDATIONS

The *total body fat content* of terrestrial mammals and probably also of insects is, in addition to other factors (s, Table 4), an important factor in toxicity testing especially of lipophilic chemicals. Therefore, it is recommended that toxicological investigations of organic chemicals with terrestrial organisms should include a determination of the total body fat content of the organisms. The above investigations have shown that the LD_{50} values of dieldrin, TCDD, and other lipophilic chemicals in different organisms are comparable if they are normalized on the total lipid content of the animals.

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REFERENCES

AHOTUPA, M., AND MÄNTYLÄ, E. (1983). Adipose tissue content as a modifier of the tissue distribution, biological effects, and excretion of a hexachlorobiphenyl in C57BL/6J and DBA/JBOM f mice. *Mol. Pharmacol.* **24**(3), 464–470.

ALBRO, P. W., CORBETT, J. T., HARRIS, M., AND LAWSON, L. D. (1978). Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on lipid profiles in tissue of the Fischer rat. *Chem. Biol. Interact.* **23,** 315–330.

ALLEN, J. R., VAN MILLER, J. P., AND NORBACK, D. H. (1975). Tissue distribution, excretion and biological effects of ^{14}C tetrachlorodibenzo-*p*-dioxin in rats. *Fd. Cosmet. Toxicol.* **13,** 501–505.

BENNETT, S. E., AND THOMAS, C. A., JR. (1963). The correlation between lipid content and percent mortality of alfalfa weevil to heptachlor and malathion. *J. Econ. Entomol.* **56,** 239–240.

BETSO, J. (1988). Dow Chemical, Midland, MI. Personal communication.

BIRNBAUM, L. S., MCDONALD, M. M., BLAIR, P. C., CLARK, A. M., AND HARRIS, M. W. (1990). Differential toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J mice congenic at the Ah locus. *Appl. Toxicol.* **15,** 186–200.

BOWMAN, R. E. (1988). University of Wisconsin, Madison, WI. Personal communication.

BRADBURY, F. R., CAMPBELL, A., AND O'CARROLL, F. M. (1958). The waxes and lipoids of resistant house flies and mosquitoes. *Indian. J. Malar.* **12,** 547–564.

BRIDGES, R. G., AND COX, J. T. (1959). Resistance of houseflies to γ-BHC and dieldrin. *Nature* **184,** 1740–1741.

CHAPMAN, D. E., AND SCHILLER, C. M. (1985). Dose-related effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J and DBA/2J mice. *Toxicol. Appl. Pharmacol.* **78,** 147–157.

DECAD, G. M., BIRNBAUM, L. S., AND MATTHEWS, H. B. (1981). Distribution and excretion of 2,3,7,8-tetrachlorodibenzofuran in C57BL/6J and DBA/2J mice. *Toxicol. Appl. Pharmacol.* **59,** 564–573.

DOMANSKI, G., AITZEITMÜLLER, K., AND JAN, E. (1974). Schnelle Fettbestimmung in Hering. Diskussion verschiedener Methoden: Ergebnisse mit einer Foss-Let-Apparatur. *Z. Lebensm. Unters. Forsch.* **154,** 129–134.

FOLCH, J., LEES, M., AND STANLEY, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509.

GAK, J. C., GRAILLOT, C., AND TRUHAUT, R. (1976). Use of the golden hamster in toxicology. *Lab. Anim. Sci.* **26,** 274–280.

GEHRING, P. J., AND BETSO, J. (1988). The Dow Chemical Co., Midland, MI. Personal communication.

GEYER, H., SCHEUNERT, I., AND KORTE, F. (1985). Relationship between the lipid content of fish and their bioconcentration potential of 1,2,4-trichlorobenzene. *Chemosphere* **14**(5), 545–555.

GREIG, J. B. (1987). Personal communication.

HARRIS, H. W., MOORE, J. A., VOS, J. G., AND GUPTA, B. N. (1973). General biological effects of TCDD in laboratory animals. *Environ. Health Perspect.* **5**, 101–109.

HENCK, J. W., NEW, M. A., KOCIBA, R. J., AND RAO, K. S. (1981). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Acute oral toxicity in hamsters. *Toxicol. Appl. Pharmacol.* **59**, 405–407.

HERBERG, L. (1987). Personal communication.

HOCHSTEIN, J. R., AULERICH, R. J., AND BURSIAN, S. J. (1988). Acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mink. *Arch. Environ. Contam. Toxicol.* **17**, 33–37.

JONES, G., AND GREIG, J. B. (1975). Pathological changes in the liver of mice given 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Experientia* **31**, 1315–1317.

KHAN, M. A. Q., AND BROWN, A. W. A. (1966). Lipids and dieldrin resistance in *Aedes aegypti*. *J. Econ. Entomol.* **59**, 1512–1514.

KODAMA, A. M. (1971). *In vivo* and *in vitro* determinations of body fat and body water in the hamster. *J. Appl. Physiol.* **31**(2), 218–222.

MATTHEWS, H. B., AND ANDERSON, M. W. (1975). Effects of chlorination on the distribution and excretion of polychlorinated biphenyls. *Drug Metab. Dispos.* **3**, 371–380.

MCCONNELL, E. E. (1987). Personal communication.

MCCONNELL, E. E., LUCIER, G. W., RUMBAUGH, R. C., ALBRO, P. W., HARVAN, D. J., HASS, J. R., AND HARRIS, M. W. (1984). Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. *Science* **223**, 1077–1079.

MCCONNELL, E. E., MOORE, J. A., AND DALGARD, D. W. (1978a). Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rhesus monkeys (*Macaca mulatta*) following a single oral dose. *Toxicol. Appl. Pharmacol.* **43**, 175–187.

MCCONNELL, E. E., MOORE, J. A., HASEMAN, J. K., AND HARRIS, M. W. (1978b). The comparative toxicity of chlorinated dibenzo-*p*-dioxins (PCDDs) in mice and guinea pigs. *Toxicol. Appl. Pharmacol.* **44**(2), 335–356.

METCALF, R. L. (1989). Insect resistance to insecticides. *Pest. Sci.* **26**, 333–358.

MORIARTY, F. (1968). The toxicity and sublethal effects of *p,p'*-DDT and dieldrin to *Aglais urticae* (L.) (Lepidoptera: Nymphalidae) and *Chorthippus brunneus* (Thunberg) (Saltatoria: Acridae). *Ann. Appl. Biol.* **62**, 371–393.

MUNSON, S. C., AND GOTTLIEB, M. I. (1953). The differences between male and female American roaches in total lipid content and in susceptibility to DDT. *J. Econ. Entomol.* **46**, 798–802.

NERI, I., ASCHER, K. R. S., AND MOSNA, E. (1962). Studies on insecticide resistant Anophelines. V. Lipid content of *Anopheles atroparvus*. *Indian J. Malar.* **16**, 41–45.

OECD (1987). *OECD Guidelines for testing of chemicals. Acute Oral Toxicity.* pp. 401, Organisation for Economic Cooperation and Development (OECD), Paris.

OLSON, J. R., HOLSCHER, M. A., AND NEAL, R. A. (1980). Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the Golden Syrian hamster. *Toxicol. Appl. Pharmacol.* **55**, 67–78.

OLSON, J. R. (1988). Personal communication.

POHJANVIRTA, R. (1988). Personal communication.

POHJANVIRTA, R., TUOMISTO, J., VARTIAINENT, T., AND ROZMAN, K. (1987). Han/Wistar rats are exceptionally resistant to TCDD (I). *Pharmacol. Toxicol.* **60**, 145–150.

POIGER, H., WEBER, H., AND SCHLATTER, C. (1982). Special aspects of metabolism and kinetics of TCDD in dogs and rats. Assessment of toxicity of TCDD-metabolite(s) in guinea pigs. In *Chlorinated Dioxins and Related Compounds. Impact on the Environment* (O. Hutzinger, R. W. Frei, E. Merian and F. Pocchiari, eds.), pp. 317–325. Pergamon Press, New York.

RAO, K. S. (1988). Dow Chemical, Midland, MI. Personal communication.

RAPP, K. (1987). Unpublished results.

REISER, R., CHADBOURNE, D. W., KUIKEN, K. A., RAINWATER, C. F., AND IVY, E. E. (1953). Variation in lipid content of the boll weevil and seasonal resistance to insecticides. *J. Econ. Entomol.* **46**, 337–340.

SCHULZ, K. H. (1987). Universität Hamburg, Hautklinik, Eppendorf. Personal communication.

SCHULZ, K. H. (1968). Zur Klinik und Ätiologie der Chlorakne. *Arbeitsmed. Sozialmed. Arbeitshyg.* **3**(2), 25–29.

SCHWETZ, B. A., NORRIS, J. M., SPARSCHU, G. K., ROWE, V. K., GEHRING, P. J., EMMERSON, J. L., AND GERBIG, C. G. (1973). Toxicology of chlorinated dibenzo-*p*-dioxins. *Environ. Health Perspect.* **5**, 87–99.

SHENG, H.-P., AND HUGGINS, R. A. (1971). Growth of the beagle: Changes in chemical composition. *Growth* **35**, 369–376.

SILKWORTH, J., MCMARTIN, D., DECAPRIO, A., REJ, R., O'KEEFE, P., AND KAMINSKY, L. (1982). Acute toxicity in guinea pigs and rabbits of soot from a polychlorinated biphenyl-containing transformer fire. *Toxicol. Appl. Pharmacol.* **65**, 425–439.

SMITH, A. H., FRANCIS, J. E., KAY, S. J., AND GREIG, J. B. (1981). Hepatic toxicity and uroporphyrinogen decarboxylase activity following a single dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mice. *Biochem. Pharmacol.* **30**(20), 2825–2830.

SPICER, S. S., SWEENEY, T. R., OETTINGEN, W. F., LILLIE, R. D., AND NEAL, P. A. (1947). Toxicological observations on goats fed large doses of DDT. *Vet. Med.* **42**, 289–293.

SPRAY, C. M., AND WIDDOSON, E. M. (1950). The effect of growth and development on the composition of mammals. *Br. J. Nutr.* **4**, 332–353.

STAHL, B. U., KETTRUP, A., ROZMAN, K. (1992). Comparative toxicity of four chlorinated dibenzo-*p*-dioxins (CDDs) and their mixture. Part I: Acute toxicity and toxic equivalency factors. *Arch. Toxicol.* **66**, 471–477.

THUNBERG, T. (1984). Effect of TCDD on vitamin A and its relation to TCDD-toxicity. In *Babury Report 18, Biological Mechanisms of Dioxin Action* (A. Poland and R. Kimbrough, eds.), pp. 333–344. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

VOS, J. G., MOORE, J. A., AND ZINKL, J. G. (1974). Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57Bl/6 mice. *Toxicol. Appl. Pharmacol.* **29**, 229–241.

WALDEN, R., AND SCHILLER, C. M. (1985). Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in four (sub)strains of adult male rats. *Toxicol. Appl. Pharmacol.* **77**, 490–495.

WALKER, M. L., SCHWARTZ, S. M., WILSON, M. E., AND MUSEY, P. I. (1984). Estimation of body fat in female rhesus monkeys. *Am. J. Phys. Anthrop.* **63**, 323–329.