

The Response of Rat Serum Lipids to Diets of Varying Composition or Contaminated with Organochlorine Pesticides

Meinrad Boll^a, Lutz W. D. Weber^{b,c} and Andreas Stampfl^b

^a Abteilung Zellchemie and

^b Institut für Toxikologie, GSF- National Research Center for Environment and Health, München, Germany;

^c Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS. 66160, U.S.A.

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The effects of different diets (high carbohydrate, high protein, high fat) and diets contaminated with polychlorinated biphenyls (PCBs) and/or γ -hexachlorocyclohexane (lindane) on the levels of serum triglycerides, cholesterol and phospholipids were investigated in Wistar rats.

Serum triglyceride levels differed significantly among the diets, while those of cholesterol and phospholipids were much less affected by the diet composition. A change in diet composition resulted in a gradual adaptation to the lipid levels characteristic of the new diet with major variations including oscillations. There was, however, no specific component of a diet that could be associated with any specific change in serum lipids. While feed deprivation decreased the serum lipids (40–65% in 3 days), refeeding the starved animals caused pronounced increases of the lipids that were different among the diets. The response of the triglyceride levels was the strongest (up to 10 times the starvation levels) followed by those of the phospholipids (4-fold) and cholesterol (2.5-fold). Response of the triglyceride levels peaked within 1 or 2 days of refeeding, whereas those of cholesterol and phospholipids took 4 days to reach the maximum.

Feeding PCB-contaminated diets increased the serum lipids in a dose-dependent manner (15–250 ppm). Higher PCB concentrations were increasingly inhibitory (350 ppm) or overtly toxic (> 400 ppm). Elevated lipids returned to the starting levels immediately after peaking (triglycerides) or only after several days (cholesterol, phospholipids) but with an earlier onset at lower PCB concentrations. Refeeding starved animals with PCB-contaminated diets also increased the serum lipids dose-dependently.

Feeding lindane-containing diets (50–150 ppm) as well as refeeding animals with lindane diets resulted in a considerable increase of the triglyceride levels, while cholesterol and phospholipids increased much less. Higher lindane concentrations (250 ppm) were inhibitory. The outcome on serum lipid levels on feeding diets contaminated with both PCBs and lindane was basically additive.

Introduction

The composition of diet exerts an important influence on serum lipid homeostasis. A diet high in sucrose will result in higher serum lipid levels than a fat-rich diet (Wilson *et al.*, 1983); glucose is more effective in raising triglyceride and cholesterol levels than sucrose (Takemoto, 1975), and sucrose is more effective than starch (Moser and Berdamer, 1974). A high protein portion in the diet

causes hypertriglyceridemia and hypercholesterolemia, but no increase of phospholipids (Hevia *et al.*, 1980), and animal protein in the diet (casein) elicits higher blood lipid levels than plant protein (soy bean) (Nagata *et al.*, 1981).

PCBs and related compounds are discussed as possible risk factors because, among others, they increase serum lipid levels in humans (Chase *et al.*, 1982; Webb *et al.*, 1986; Hirota *et al.*, 1993). This effect has been observed also in rats (e.g. Matthews *et al.*, 1984). Studies in animals were performed with different industrial PCB mixtures consisting mainly of various isomers of tri- to hexachlorobiphenyls, yet they all resulted in a significant elevation of triglyceride and cholesterol

Reprint requests to Dr. Meinrad Boll, Abt. Zellchemie, GSF, Forschungszentrum f. Umwelt & Gesundheit, München, Geb. 57, Postfach 1129, D-85758 Oberschleissheim.
Fax: 089–3187–3449.

levels: Phenoclor (DP6, 60% Cl: Narbonne *et al.*, 1978; DP5, 50% Cl: Poul, 1991), Clophen A50 (54% Cl: Baumann, 1979; present paper), Aroclor 1248 (48% Cl: Quazi *et al.*, 1983; Oda *et al.*, 1994), Kanechlor 500 (50% Cl: Yagi *et al.*, 1985) or even 2,3,4,7,8-pentachlorodibenzofuran (Brewster *et al.*, 1988). For a characterization of PCB mixtures see Lang (1992). A finding that awaits explanation is that 2,2',4,4',5,5'-hexachlorobiphenyl and 3,3',4,4'-tetrachlorobiphenyl caused significant increases of triglycerides and cholesterol only in liver tissue, but not in the serum of rats (Azaïs-Braesco *et al.*, 1990). The hyperlipidemia found after PCB exposure can be influenced by the composition of diet: a high protein diet exacerbates the problem (Kato *et al.*, 1980) whereas high fiber (Quazi *et al.*, 1983) or antioxidants in the diet (Kato *et al.*, 1981; Oda *et al.*, 1987; Yamamoto *et al.*, 1994) lower lipid levels.

Lindane (γ -hexachlorocyclohexane), an insecticide widely used to fight topical insect infestation in humans, can be absorbed transdermally and gives rise to acute toxicity mostly to the central nervous system (Mortensen, 1986). It has recently been demonstrated (Boll *et al.*, 1995) that it also affects hepatic lipogenesis. In this study we have attempted to investigate the effects of diets of varying composition as well as diets contaminated with PCBs, with lindane, or with both highly lipophilic xenobiotics together on blood lipid levels in order to better characterize possible health hazards posed by unbalanced or xenobiotic-tainted diet.

Experimental

Male Wistar rats, 30–35 days old, from our own husbandry were used. They were routinely kept on a standard rodent chow (diet 1 of Table I) on a 12h light/dark cycle (lights on: 6 am to 6 pm) in a humidity-controlled room at $25 \pm 2^\circ\text{C}$. The animals received this diet for at least 14 days before they were weighed and used for the experiments.

Diets containing polychlorinated biphenyls (PCBs) and/or hexachlorocyclohexane (lindane) were prepared by slowly adding the pesticide, dissolved in ethanol, under stirring to powdered standard rodent chow (50 ml ethanol per 500g diet). The mixture was then pasted with water, pellets were formed and dried at 25°C for 3 days. Control diets for the pesticide experiments were prepared as described but with ethanol alone. The term

PCB-free or lindane-free stock diet refers to these diets. Xenobiotic concentrations higher than 250 ppm for PCBs and 350 ppm for lindane were normally not used. Up to these concentrations the treated rats maintained their weight relative to the control animals.

Blood was obtained from ether-anesthetized animals by cardiac puncture. Serum lipids were analyzed using commercial diagnostic kits: triglycerides (Sigma # 320 A), total cholesterol (Boehringer 1442341) and phospholipids (Boehringer 691844). The levels of the serum lipids are expressed as g/l.

The standard rodent chow (tpf 1324) was obtained from Altromin, D-49828 Lage, Germany. The specified diets (diets 2–6 in Table I) were obtained from ICN Nutritional Biochemicals, Cleveland, Ohio, U.S.A. The composition of all diets is listed in Table I. Clophen A-50 (source of polychlorinated biphenyls) was a product of Bayer, Leverkusen, Germany. It is a mixture of polychlorinated biphenyls with a chlorine content of 54% and an average molecular weight of 327. A concentration of 100 ppm in the diet corresponds to approximately $0.30 \mu\text{mol PCB/kg chow}$. For its components see Oesterle and Deml (1984). The commercial diagnostic kits were purchased from Boehringer, Mannheim, Germany or from Sigma Chemical Company, St. Louis, U.S.A. (D-82041 Deisenhofen). Lindane was also obtained from Sigma.

Results and Discussion

Diets of different composition affect serum lipid levels

The different levels of the serum lipids (triglycerides, cholesterol and phospholipids) that resulted from feeding diets of different composition are summarized in Table I (together with the basic compositions of these diets). The levels in animals fed the standard rodent chow (diet 1), the routinely employed diet, represented the basic levels in this investigation. Triglycerides were most of all affected by the diet composition: they increased by about 50 % upon feeding of diets 2 and 4, doubled with diets 5 and 6, and almost tripled with diet 3. The levels of cholesterol and phospholipids showed little dependence on the composition of diet (with the exception of diet 6). None of the three basic components of the diets-carbohydrate, protein and fat-appeared solely responsible for the elevation of serum triglycerides. Diets 5 and 6

Table I. Basic levels of serum lipids in rats fed diets of different composition.

The standard rodent chow (diet 1) contained 61% cereal-derived carbohydrate, 19% protein (extracted from soy bean grist), 7% fat (soy bean), 5% fibrous material, 3% minerals and vitamins and 5% ashes. Experimental ICN diets (diets 2–6) contained casein as source of protein, cotton seed oil as fat and corn starch (diets 5 and 6). They also had 1.5% brewers yeast and 4% salt- and vitamin mixture. Diet 2, in addition, contained 10% fibrous material. Animals, approximately 30d-old, received the indicated diet for 18–20 days before the serum lipid levels were determined. Values are means of 6 animals \pm SEM.

Diet composition (ingredients in percent)	Triglycerides	Serum lipids [g/l] Cholesterol	Phospholipids
1 carbohydrate 61 protein 19 fat 7	0.95 \pm 0.092	0.75 \pm 0.072	1.40 \pm 0.142
2 sucrose 64 protein 21 fat 0	1.47 \pm 0.14	0.85 \pm 0.085	1.32 \pm 0.130
3 sucrose 68 protein 18 fat 8	2.7 \pm 0.265	0.82 \pm 0.080	1.40 \pm 0.141
4 sucrose 22 protein 64 fat 8	1.47 \pm 0.145	0.80 \pm 0.081	1.34 \pm 0.133
5 starch 58 protein 27 fat 10	2.19 \pm 0.22	0.84 \pm 0.082	1.35 \pm 0.132
6 starch 76 protein 8 fat 10	1.98 \pm 0.19	1.14 \pm 0.11	1.80 \pm 0.185

were similar to diet 1 with respect to their carbohydrate and fat portions but diet 5 contained much more protein (27%) whereas diet 6 was low in protein (8%). Yet switching to either diet 5 or 6 resulted in doubled serum triglyceride levels as compared to diet 1 (19% protein). Diet 2, a high-sucrose, fat-free diet, resulted in practically identical triglyceride levels as diet 4, which was low-sucrose, high-protein, with normal fat content. This suggests that both a high protein and a high sucrose portion in the diet can contribute to high serum triglycerides, whereas a comparison of diets 2, 3 and 6 indicates that the fat content of the diet exerts little influence on serum lipid levels. Diet 3 differed from the standard diet 1 only in the nutritional availability of the carbohydrate, viz. sucrose vs. cereal-derived carbohydrate, but produced three times higher triglyceride levels with essentially no effect on cholesterol and phospholipids. Finally, diet 6, low in protein and relatively high in fat, as compared to diet 1, caused an appreciable elevation of both cholesterol and phospholipids.

The data shown here were obtained with young male Wistar rats. It has previously been reported that the levels of serum lipids were similar in young (30d-old) male and female animals. The levels remained constant with increasing age in males, but increased considerably in females, reaching levels three times higher (triglycerides) and twice (cholesterol and phospholipids) those of adult (160d-old) males (Boll *et al.*, 1980). A comparison of the serum lipids in adult animals of both sexes fed with the diets of Table I also revealed considerably higher levels in females than in males (data not shown).

Changes of serum lipids in response to dietary variations

When, during feeding the rats, the diet was replaced by another diet of different composition, serum lipid patterns adjusted to levels characteristic for the new diet. Fig. 1 shows the time course of this adaptation on transition from the standard

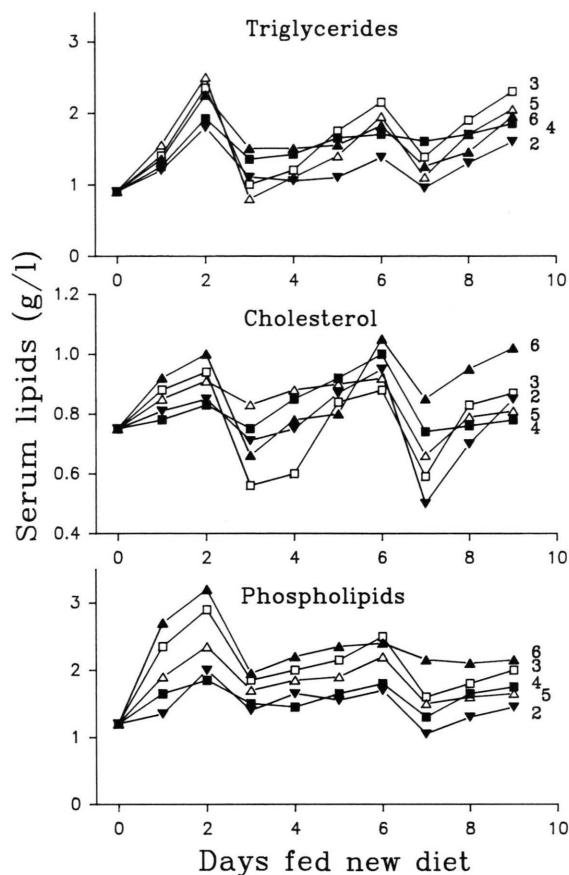


Fig. 1. Effect of changes in diet composition on the levels of serum lipids. Animals fed standard rodent chow (diet 1 of Table I) were switched to the new diet at time zero. Numbers indicate the diets of Table I. Values are means of 6 animals. SEM (7–13%) omitted for clarity.

rodent chow (diet 1) to the five experimental diets of Table I. The process of adaptation was time-consuming and, as can be seen from Fig. 1, it still was not yet completed after 9 days of feeding a new diet. The values depicted in Table I are the levels that were attained after 18–20 days of feeding the respective diets and they represent definite serum levels that remained constant during continuous feeding. The patterns of adaptation were the same for all diets; differences existed only in the degree of change induced by a different dietary composition. The initial response to any change of diet was a strong increase of serum lipid levels, lasting for 2 days and reaching levels close to three times normal (triglycerides). There followed a precipitous decline to levels around or

even less than normal, then another, slower increase to about twice normal levels, which was followed by another decline and another increase, each with a frequency of 3 to 4 days. Diet 3, high sucrose, normal fat, was particularly effective in eliciting these oscillatory responses with the triglycerides, whereas the fat-free diet 2 was least effective (Fig. 1, upper panel). The patterns of adaptation of serum cholesterol and serum phospholipids were essentially identical, but less pronounced than those of serum triglycerides. The first peak, after two days of feeding a new diet, was little conspicuous in the case of cholesterol, but more obvious with the phospholipids. In contrast the following peak, at six days, was more pronounced with cholesterol and hardly noticeable with the phospholipids. Diet 6, containing starch as carbohydrate and relatively high fat, was most effective in causing overshooting responses of serum cholesterol and phospholipid levels (Fig. 1, center and bottom panels).

Oscillations as shown in Fig. 1 have been reported previously to occur in the activities of key enzymes of hepatic and adipose tissue lipogenesis (Boll *et al.*, 1994 and references therein). It is speculated that the oscillating responses of serum lipids shown in Fig. 1 are a reflection of the activity changes of the synthesizing enzymes. There is currently no mechanistic explanation as to the origin of these oscillations. Also no explanation exists concerning the elevated serum lipid levels after 2 days feeding a new diet (Fig. 1). Moser and Berdarnier (1974) have reported that switching rats from a starch-containing to a sucrose-containing diet, or vice versa, resulted in elevated serum cholesterol and triglyceride levels at 50 days after the beginning of the new diet, but no longer at 92 days after switching of the diet. The fact that the animals in the present study were sacrificed shortly after the end of their nocturnal feeding period might suggest lipidemia as the reason for the findings; however, the normal range triglyceride level of around 0.9g/l also reflects the postprandial state. One possible cause for these differences might be that diet 1 which the animals had received before was a standard commercial diet, whereas diets 2 to 6 were semipurified diets containing components that have a more immediate effect on serum lipids. It has been shown that a diet made from defined components (e.g., casein for protein, olive oil for

fat, etc.) can affect the toxicity of PCBs in both rats and mice (Yagi *et al.*, 1985 and references therein). From a nutritionist's standpoint it would be of great importance to exactly know which nutritional components affect lipid homeostasis.

Starvation/refeeding response

It is well known that levels of serum lipids are influenced by the amount of feed intake; for example, restriction to 70% of voluntary feed intake for one year will result in lower levels of serum triglycerides and cholesterol in rats (Cleary *et al.*, 1987). Using young male Wistar rats, serum triglyceride, cholesterol and phospholipid levels decreased approximately 65, 45 and 40 percent, respectively, after 3 days of feed deprivation (Fig. 2, dashed lines). The decline was smaller on shorter periods of starvation. Refeeding the starved animals with various diets resulted in a strong increase of serum lipid levels. In the case of triglycerides (Fig. 2, top panel) the increase peaked after 1 day and at 5- to 12-times the starvation levels. The standard rodent chow (diet 1) was least effective in producing a response, whereas diets 3 and 5 were most effective. Within another two days of refeeding serum triglyceride levels were back to nearly the prestarvation levels, and then began to increase again, suggesting that oscillations similar to those in Fig. 1 would follow.

The levels of cholesterol and phospholipids responded much more slowly; they reached their maximum not before day 3 and 4. No indication of return to lower levels was evident after 5 days of refeeding (Fig. 2, center and bottom panels). As with the triglycerides the standard rodent chow (diet 1) was least effective, with cholesterol and phospholipid levels stalling or even decreasing on the first day of refeeding, and just reaching prestarvation levels by day 5. Such decrease of the cholesterol and phospholipid levels on the first day of refeeding following starvation has already been described (Boll *et al.*, 1985). Diets 3 and 6 again were most effective (cf. Fig. 1), inducing almost three times starvation levels of serum cholesterol and more than 4 times the starvation levels of phospholipids. The high-protein, low-carbohydrate diet 4 was only slightly more effective than the standard rodent chow (diet 1) with all three serum lipids.

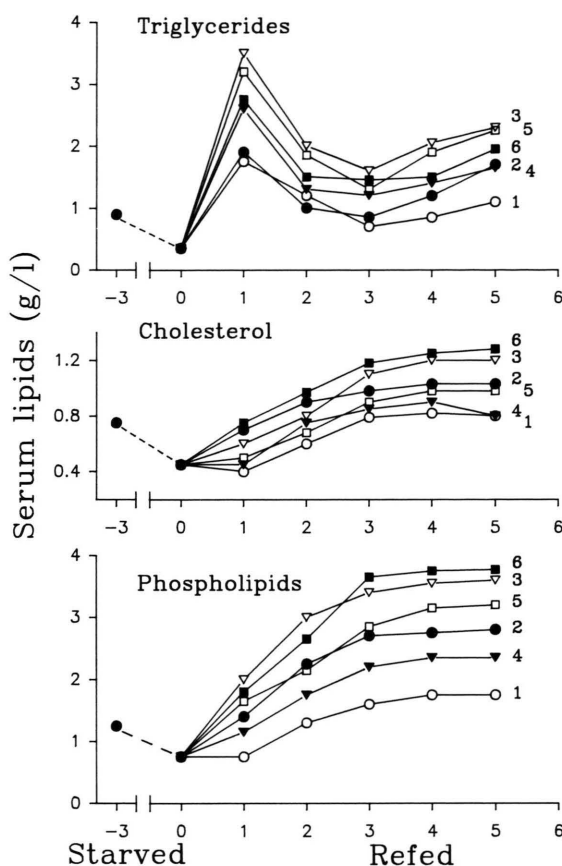


Fig. 2. Changes of serum lipids in response to refeeding starved animals with different diets. Animals were kept on the standard rodent chow before feed was withdrawn for 3 days. The animals were then refed with the different diets. Numbers indicate diets of Table I. Values are means of 6 animals. SEM (7–15%) omitted for clarity.

The response of the serum lipids to starvation and also to subsequent refeeding was more profound in 30d-old animals than it was in 160d-old animals. Serum triglycerides, cholesterol and phospholipids in adult Wistar rats decreased only 36, 23 and 18 percent, respectively within 3 days of starvation (Boll *et al.*, 1985). This would be consistent with data reported by Freedland (1967) and by Boll *et al.*, (unpublished data), where, upon starvation, hepatic activities of lipid-synthesizing enzymes declined much more in young rats than in adult ones. In the present investigation there were no significant differences between animals fed the different diets of Table I in the extent of the starvation-induced decline of the serum lipids. The

increase of the serum lipids upon refeeding starved animals also was much more pronounced in young than in adult animals. Increase for the three serum lipids was 70–100 % greater in young individuals, with triglycerides being most of all affected (Boll *et al.*, 1985). Likewise in response to refeeding the activities of all lipogenic enzymes investigated increased considerably more in young animals than in older ones (Boll *et al.*, 1982).

Effect of polychlorinated biphenyls on serum lipids

PCBs in the diet caused a dose- and also time-dependent increase of serum lipids (Fig. 3). Tri-

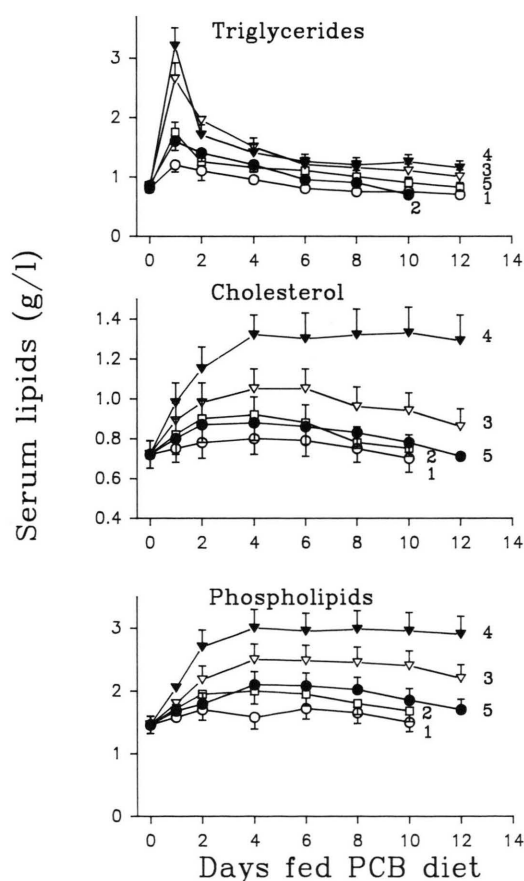


Fig. 3. Effect of PCB-containing diets on the levels of the serum lipids. Animals were kept on the standard rodent chow; feeding with PCB-containing diets was initiated at time zero. Dietary PCB levels were (1) 15ppm; (2) 60ppm; (3) 120ppm; (4) 250ppm; (5) 350ppm, respectively. Controls received the PCB-free stock diet (see Experimental). Values are means of 4 animals \pm SEM. Values of controls fed the PCB-free stock diet were constant and were omitted for clarity.

glyceride levels peaked 1 day after the onset of PCB-feeding (top panel). They were slightly elevated with the lowest dose, 15 ppm Clophen A50 in the diet (curve 1), and reached a maximum with 250 ppm at about 400 % of untreated controls (curve 4). Due to overt toxicity, as evidenced by reduced feed intake and body weight gain of the treated animals, 350 ppm PCBs in the diet resulted in a much lower triglyceride level (curve 5). Serum levels of cholesterol and phospholipids responded more slowly and less pronounced to the PCB intoxication (Fig. 3, center and bottom panels). While serum triglycerides, elevated by PCB treatment returned towards the levels found in naive controls immediately after the peak (top panel), those of cholesterol and phospholipids remained at high levels (for several days with the highest dose) before beginning to return towards control levels (center and bottom panels). In any case the return occurred despite the continued intake of PCBs, but with an earlier onset at the lower PCB concentrations.

When animals which had been deprived of feed were refed with PCB-poisoned diets the levels of the serum lipids also increased in relation to the PCB dose in the diet (Table II). The pattern of the response was essentially the same as seen after starvation and refeeding diets of different composition (Fig. 2, see curve 1 for standard rodent chow) with peak triglyceride levels after refeeding PCB-contaminated diets being reached within 1

Table II. Increase of serum lipids in response to refeeding starved animals with diets containing different amounts of PCBs.

Animals were kept on the standard rodent chow before feed was withdrawn for 3 days. Following starvation animals were refed with the standard rodent chow containing different amounts of PCBs. Controls were refed with the PCB-free stock diet (see Experimental). Values are increase of the serum lipids in percent over the controls refed with the PCB-free stock diet at the time of maximal increase (day 1 for triglycerides and day 4 for cholesterol and phospholipids) (see Fig. 2, curves 1). Values of 4 animals \pm SEM.

PCBs (ppm)	Serum lipids (percent increase)		
	Triglycerides	Cholesterol	Phospholipids
15	8.2 \pm 0.83	5.3 \pm 0.52	5.9 \pm 0.60
30	15.2 \pm 1.40	10.4 \pm 1.10	8.1 \pm 0.80
60	35.1 \pm 3.60	15.6 \pm 1.58	13.4 \pm 1.35
120	41.9 \pm 4.22	23.4 \pm 2.38	22.2 \pm 2.25
250	45.6 \pm 4.50	30.2 \pm 3.10	29.8 \pm 3.00
350	39.1 \pm 3.80	26.3 \pm 2.65	25.1 \pm 2.50

day, and peak levels of cholesterol and phospholipids by 4 days. Triglycerides peaked at levels 45% above those of the controls (starved and refed without PCBs) and cholesterol and phospholipids reached levels 30% higher than controls. 350 ppm Clophen A50 in the diet caused a minor inhibition. A comparison of Fig. 3 and Table II reveals that the response of serum lipids to starvation/ refeeding a PCB-contaminated diet was not as pronounced as the response to administering the same diet to fed animals.

Effect of lindane on serum lipids

Lindane also affected the serum lipids in a dose-dependent manner (Table III). Feeding a diet containing 150 ppm lindane for 2 days caused moderate increases of serum triglycerides (+90%), cho-

lesterol (+30%) and of the phospholipids (+20%); (Table III A). A small increase was seen for the triglycerides at 50 ppm lindane while at 350 ppm in the diet all three lipids were considerably below control levels although this dose produced no overt signs of toxicity. It has recently been demonstrated that the response of serum triglyceride to lindane peaked at two days of feeding a 150 ppm lindane-containing diet, then decreased again strongly but remained elevated for at least another 9 days; cholesterol and phospholipid levels were slightly increased after 6 and 8 days of lindane feeding, respectively (Boll *et al.*, 1995) (see also determinations of serum lipids in Table III after 2 and 6 days, respectively). When the exposure to lindane-contaminated feed was preceded by a 3-day period of starvation 50–150 ppm lindane in the diet resulted in an increase of serum lipids. At 150 ppm no higher increase than that at 100 ppm was to be seen and 250 and 350 ppm both inhibited the effect, causing a decrease of serum lipids below the levels of the control animals that were starved and then refed the lindane-free stock diet for 2 days (Table III B).

There appears to be only one publication concerning plasma lipid levels in rats after lindane exposure. Andrews and Gray (1990) report that after exposure to lindane at 10 or 20 mg/kg/day for 10 weeks serum cholesterol was increased in Long-Evans rats, whereas serum triglycerides were lower. These results could be in keeping with the present ones, as the Wistar rats were exposed to similar doses (roughly 20 to 45 mg/kg/day) and serum triglyceride levels fell after their initial rise, whereas those of cholesterol kept rising over a period of one week (Boll *et al.*, 1995). Using non-toxic doses of lindane Ishikawa *et al.* (1978) did not find changes in the levels of serum α -lipoprotein cholesterol in rats, whereas an epidemiologic study in humans seems to indicate that lindane exposure does increase α -lipoprotein cholesterol (Carlson and Kolmodin-Hedman, 1977). Lindane does exert an effect on lipid synthesis in rat prostate (Gutierrez-Ocana *et al.*, 1989), testes (Chowdhury *et al.*, 1990) and liver (Boll *et al.*, 1995). In the case of lipogenetic enzymes of rat liver it was found that doses of 50 and 150 ppm lindane in the diet caused a stimulation of activities, whereas 250 and 350 ppm, respectively, caused inhibition (Boll *et al.*, 1995). This effect of lindane on hepatic lipo-

Table III. Effect of lindane on serum lipids under different feeding conditions.

Animals were fed the standard rodent chow before dietary manipulations were initiated at time zero. Panel A: Animals received diets containing the indicated lindane concentrations. Controls received the lindane-free stock diet (see Experimental). Triglycerides were determined after 2 days, cholesterol and phospholipids after 6 days of feeding. Levels of controls remained constant. Panel B: Animals were deprived of feed for 3 days and subsequently refed for 2 days with the lindane-free stock diet or with diets containing different concentrations of lindane. Values are means of 4 animals. SEM (7–12%) omitted for clarity.

Treatment of animals	Triglycerides	Serum lipids [g/l]	
		Cholesterol A	Phospholipids
Control feeding lindane diet (ppm)	0.95	0.73	1.35
25	0.96	0.70	1.35
50	1.25	0.75	1.30
100	1.50	0.80	1.40
150	1.85	0.95	1.63
350	0.84	0.70	1.25
B			
Control starved (3 days)	0.95	0.73	1.35
refed (2 days)	0.32	0.42	0.75
refed (2 days) + lindane (ppm)	1.95	0.83	1.45
50	2.15	0.92	1.62
100	2.80	1.05	1.84
150	2.85	1.10	1.92
250	1.52	0.45	1.10
350	1.20	0.30	0.85

genesis can explain the present findings on serum triglyceride levels, but it is not known by which mechanism lindane can affect the enzymes.

A comparison of the responses of the two pesticides, PCBs and lindane, on the serum lipids revealed a similarity of the kinetics but a difference of their effectiveness. Triglycerides responded most pronounced to either compound causing a strong but short-lived increase with a peak within 1–2 days while cholesterol and phospholipids always responded much slower and less pronounced. However, the serum lipids were more sensitive to PCBs (already responding at 15 ppm) than to lindane (responding not before 50 ppm). The highest concentrations resulting in an increase of the lipid levels were at 250 ppm with the PCBs but slightly less than 200 ppm with lindane.

Combined effect of polychlorinated biphenyls and lindane on the serum triglycerides

This experiment was conducted to find out if the combined effects of both xenobiotics on serum lipid levels would turn out additive or possibly even synergistic. As Fig. 4 demonstrates the combined effect of 75 ppm Clophen A50 and 100 ppm lindane in the diet on the serum triglycerides had a clearly additive effect when the rats were ad libitum-fed before onset of the pesticide exposure (upper panel). When the animals were starved for 3 days before exposure to the combined pesticides the triglycerides increased significantly (Fig. 4, lower panel, curve 1). The increase was inhibited by 250 ppm lindane (2) but stimulated by 75 ppm PCBs (3). As the changes were opposite with respect to the pesticide-free stock diet refed controls they actually cancelled each other out (4). In both cases the maximum of response was seen on the first or second day of feeding the contaminated diet, with the levels reverting toward normal on the following days of continued exposure. The explanation for this unusual result of lindane exposure seems to emerge from lindane's effect on hepatic enzymes of lipogenesis. When ad libitum-fed rats were exposed to a lindane-contaminated diet the activities of several key enzymes fell precipitously on the first day, but began to recover immediately thereafter. When the animals were starved for three days before lindane exposure, however, a differential effect was observed: with low lindane

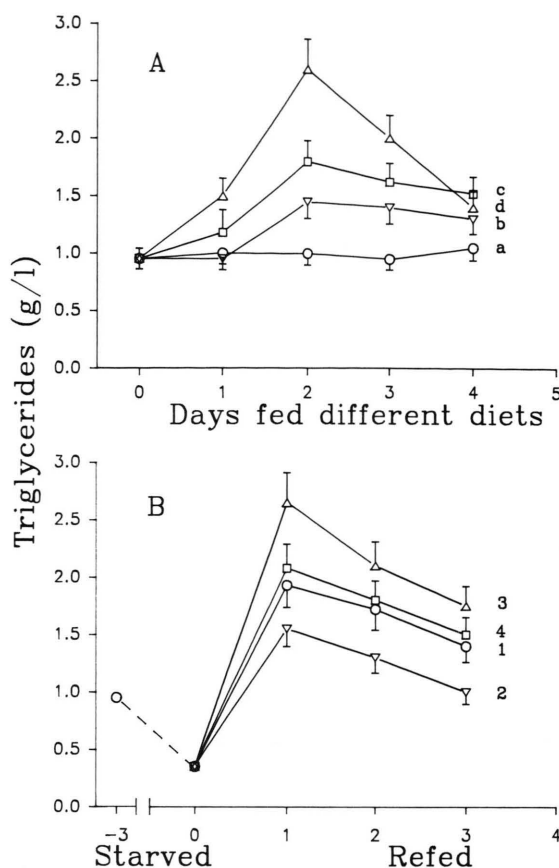


Fig. 4. Combined effect of lindane and PCBs on serum triglycerides. Animals were kept on the standard rodent chow before dietary manipulations were initiated. Panel A: At time zero the animals were switched to diets containing either 100 ppm lindane (b), 75 ppm PCBs (c) or 100 ppm lindane plus 75 ppm PCBs (d). Controls continued to receive the pesticide-free stock diet (a) (see Experimental). Values are means of 4 animals \pm SEM. Panel B: Animals were starved for 3 days and then refed with the pesticide-free stock diet (1) or with this diet containing 250 ppm lindane (2), 75 ppm PCBs (3) or 250 ppm lindane plus 75 ppm PCBs (4). Values are means of 4 animals \pm SEM.

doses enzyme activities displayed an overshoot beyond the response to standard chow, but this response was increasingly blunted with higher doses of lindane (Boll *et al.*, 1995). Given the present state of knowledge it seems premature to decide whether overexposure with lindane, which occurs quite frequently in the course of treatment for pest infestation, poses a major hazard with respect to lipid homeostasis.

The changes in serum lipid levels in response to pesticide action which are reported here are most likely due to the action of PCBs and/or lindane on hepatic enzymes of lipogenesis (Boll *et al.*, 1994; 1995). This is a direct effect on the hepatocyte, as the changes in the activity of the lipogenic en-

zymes can be reproduced *in vitro* using PCB-treated hepatocytes (Boll *et al.*, 1994). Additional studies are necessary to characterize the mechanism of these pesticide actions at the molecular level.

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