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Research Article

Dose–response feeding study of short chain chlorinated paraffins (SCCPs) in laying hens: Effects on laying performance and tissue distribution, accumulation and elimination kinetics

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Technical short chain chlorinated paraffins (C10–C13 with 60% chlorine) were fed to 93 laying hens from 24 to 32 weeks of age in increasing concentrations of up to 100 mg/kg feed. No significant influence on health, relative organ weights or performance (laying intensity, egg weight, feed consumption) was noted. The chlorinated paraffin content of the tissues was linearly related to the concentration of short chain paraffins of the feed. The highest concentrations were found in abdominal fat, egg yolk and fatty tissues. Breast muscle, egg albumen and bile fluid contained minimal or no residues. Less than 1% of the chlorinated paraffins ingested were incorporated into the body (without head, feet, gut and feathers), whereas about 1.5% were eliminated with the egg yolk and 30% were excreted with urine and faeces. A six-week kinetic depuration study revealed a biphasic elimination with half-lifes of 4–40 min (liver, kidneys, legs, fat, blood) for the initial rapid phase, and 15–30 days (blood, fat, liver, yolk, kidneys, legs) for the terminal slow phase.

Keywords: Carry over / Chlorinated paraffins / Depuration study / Egg / Laying hen

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1 Introduction

Chlorinated paraffins are formed by the chlorination of straight chain paraffins. The technical products are marketed under a variety of names [1]. They are a very complex mixture with different chain lengths and degrees of chlorination. Products with a chain length between C10 and C13 are classified as short chain chlorinated paraffins (SCCPs), which are more toxic than medium and long chain products and are characterised as possibly carcinogenic to humans [2, 3]. The EU restricts the use of SCCPs in metal-working fluids and leather-finish products [4]. Chlorinated paraffins are used worldwide as plasticisers in plastics (PVC), additives in metalworking fluids, flame retardants and additives in paints. In Europe, the annual consumption ranges from 100 to 200 kt and in Germany, during 1990/1991, 20–30 kt were used [5, 3]. In 1995, the production of SCCPs was stopped in Germany, and other EU member states also significantly reduced the amount used to about 4 kt in 1998 [6]. The substitution of chlorinated paraffins (CPs) as flame retardants, additives in paints, coatings and sealants will, however, take time, and the international production is still going on, especially in Asian countries [6]. The widespread and uncontrolled use of the CPs, their release into the environment and their stability lead to environmental contamination [3, 6, 7]. Chlorinated paraffins were detected in the air, water, sediments, in sewage sludges of waste water treatment plants [8–10] and in household wastes [6]. High concentrations, and also no residues, were analysed in sewage sludge as well, both from industrial and rural areas [10, 11]. It was suggested that CPs may be present in some household products and thus enter the wastewater [10]. As a result of using sewage sludge as fertiliser on fields, the CP contamination may extend to nonindustrial areas. Also, remote areas may be affected by transportation of the contaminant *via* water, wind and rain. Residues have not only been detected in mussels and fish, but also in terrestrial animals

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Abbreviations: BCF, bioconcentration factor; **CP,** chlorinated paraffin; **SCCPs,** short chain chlorinated paraffins

and humans [12, 13]. Farm animals may be affected and their products contaminated through contact with soil inputs of sewage sludges and by ingestion by organisms living in the soil [9]. More than 50% of the 2.7 million tons (d.m.) of the sewage sludge produced in Germany were used in agriculture [14]. Information about the (agricultural) food chain and human exposure due to the ingestion of products contaminated by chlorinated paraffins is scarce.

Therefore, an eight-week experiment was conducted by feeding SCCPs in increasing concentrations, up to 100 mg/kg feed to laying hens, to test the influence of CPs on the health and production rate of the animals and the possible tissue retention of chlorinated paraffins. After the exposure, the animals of the 100 mg/kg group received uncontaminated feed for six weeks to follow the elimination kinetics in the tissues.

2 Material and Methods

2.1 Chemicals

SCCPs with 60% chlorine were obtained from Fluka (Sigma-Aldrich, Taufkirchen, Germany). Technical-grade acetone, *n*-hexane, cyclohexane and ethyl acetate were distilled before use. All other chemicals were of laboratory reagent grade.

2.2 Animals and procedures

Laying hens of the 'Lohmann selected Leghorn' strain, aged 18 weeks, were kept in single cages and fed a commercial layer diet until the commencement of the experiment after six weeks. Fourteen days before the start of the experiment all birds were weighed. 93 birds were randomly allocated to six treatments. The diets of the six groups were supplemented with CP in the concentrations 0, 2, 20, 45, 70 and 100 mg/kg feed (Groups I–VI). Groups I and VI consisted of 21 and 36 animals, respectively, 36 animals were distributed equally to the Groups II–V. To follow up the decrease of CP concentrations in the tissues, and to describe the elimination kinetics, a withdrawal study was started with 15 birds of Group VI at the end of experiment (8th week of the experiment). CPs were dissolved in soy oil and added as 0.8% premix to the diet. The hens had *ad libitum* access to feed and water. The commercial diet contained wheat, corn, soy bean meal (steam heated), calcium carbonate, sunflower oil meal extract, vegetable oil, green meal, minerals and vitamins (calculated composition: 17% crude protein, 5% crude fat, 4.5% crude fibre, 12.5% crude ash, 3.5% calcium, 0.55% phosphorus, 0.36% methionine, 11.4 MJ metabolizable energy/kg).

The feed consumption of all animals of Groups I and VI was recorded weekly. Laying performance was calculated over two periods of 28 days for these groups. The egg weights for Groups I and VI were calculated as the mean weights of eggs collected for 4 days in the fourth or eighth week of experiment. Birds of 32 weeks of age of all groups were weighed. Nine birds, each of Groups I–VI, were killed by cutting the neck vessels after manual stunning. The inner organs of nine birds *per* group (Groups I–VI) were dissected, weighed and related to the live weight of the individual birds.

At the end of the experiment (32 weeks of age), tissue samples were pooled from nine animals of the Groups I–VI. A mixed sample of eggs (yolk and albumen) from nine animals of each group was prepared. Excreta from five birds of each group were collected in the morning, after a 24 h sampling, and mixed. Feed samples of all six diets were taken at three times during the eight-week experiment. During the withdrawal study, two additional samples of the uncontaminated feed were analysed for CP to confirm the CP content of the Control.

To follow the increase in CP concentration in eggs, samples of yolk and albumen pooled from ten eggs from different birds of the Control Group (Group I) and Group VI were analysed on nine specific dates from the beginning until the end of the experiment.

The withdrawal study started after the termination of the dose–response feeding experiment and lasted from 32 to 38 weeks of age of the hens. Twenty-four hens of Group VI received the uncontaminated diet. On days 1, 2, 4, 8, 14, 21, 28 and 42 after the study started, three animals each were slaughtered, the tissues or organs removed and mixed for analysis. On days 14, 28 and 42, three animals each of Group I served as a control. Samples of yolk and albumen were pooled from six to ten eggs. Yolk (albumen) was sampled on days (1), (2), 4, (4), 7, (9), 17, 21 and 40 (40, 42), after the beginning of the withdrawal study. On days 9, (1 and 4), yolk (albumen) samples of the Control Group were collected.

All collected physiological samples were pooled, homogenised by an Ultra-Turrax™ and stored at –20°C before being analysed.

Ethical approval of the experiments was obtained from the Bezirksregierung Lüneburg.

2.3 Analyses

The same GC-analysis method and instrument settings as described previously [15], were applied using a short GC capillary connected with an EC-detector which eluted the CPs in a single unresolved peak with a retention time (end of the peak) of up to 10 min. The short elution time rendered a relatively sharp peak with sufficiently low detection limits of 0.01–0.1 mg CP *per* kg at sample weights of 10–1.5 g. For quantification, a technical short-chain (C10–C13) CP mixture from Fluka with 60% chlorine was employed as an external analytical standard. The standard curve was linear in the range of 0.5–25 ng CP/μL. The results are given as 'total CP'. Since an identical technical CP mixture from the

same manufacturer was added to the feed, a corresponding carbon chain length and chlorine content in samples and standard can be assumed, so the basis for quantification is given [16]. Furthermore, no indications of interfering substances, such as toxaphene or PCBs, were found in the feed, which made careful additional separations unnecessary [17].

Physiological samples were homogenised using an Ultra-Turrax™, centrifuged and cleaned up prior to analysis as described in [15]. Analytical results were related to wet weight and are at least the means of duplicate analyses of pooled samples. The RSDs of repetitions were 9% (yolk), 17% (fat), 18% (feed) and 20% (liver) or 3% (feed), 5% (fat), and 12% (yolk) for concentrations of 0.1–1.0 or >1.0–15 mg/kg. The mean recoveries for feed ($n = 8$) and tissues (muscle, blood, yolk, albumen, fat, liver, kidneys, excreta; $n = 2$–5) were 79–106% using fortification levels of 0.7–3.8 mg CP *per* kg. The recoveries were 84 and 97% for fat ($n = 5$) and bile fluid ($n = 3$) at 5 and 11 mg CP *per* kg or 12 and 23 mg CP *per* kg additions.

2.4 Statistics

A one-way factorial ANOVA procedure was used to analyse the performance and organ weight data. A linear regression was performed, relating the CP concentration in various tissues and eggs to the CP concentration in feed.

The kinetics of CP elimination from the hen's body after withdrawing the CP-supplemented diets followed a biexponential course and the respective data were fitted to a two-compartment model:

$$y = A\mathrm{e}^{-\alpha t} + B\mathrm{e}^{-\beta t} \quad (1)$$

where y = CP is the concentration at time t (mg chlorinated CP *per* kg wet weight), A, B are the initial concentrations at $t = 0$, α, β are the rate constants of initial and terminal elimination phase, and t is the time (day). The half-lifes were calculated as $t_{1/2}\alpha = \dfrac{\ln(2)}{\alpha}$ for invasion or initial elimination phase and as $t_{1/2}\beta = \dfrac{\ln(2)}{\beta}$ for the terminal elimination phase.

The CP residues in egg yolks were fitted to a combined model to cover not only the disappearance from yolks after CP withdrawal, but also the preceding enrichment phase after the introduction of the CP-diets:

$$y = C(1 - \mathrm{e}^{-c1t})(d < 50) + (A\mathrm{e}^{-\alpha t} + B\mathrm{e}^{-\beta t})(d \geq 50) \quad (2)$$

where y, t, A, B, α and β are the same as for the model described by Eq. (1), and C is the asymptotic maximum CP enrichment of eggs and $c1$ is the enrichment rate constant.

The nonlinear curve fitting module of Statistica for the Windows™ operating system (StatSoft Inc., 1994) was used for fitting the data to the models.

3 Results and discussion

3.1 Feed intake and laying performance

The birds were fed with diets artificially contaminated with SCCPs (C10–C13), in increasing concentrations over a 50-fold range from 2 mg/kg up to 100 mg CP *per* kg feed. None of the 93 birds, of which 36 received the highest CP additions of 100 mg/kg (Group VI), died in the eight-week experiment. There were no indications that even the highest additions of chlorinated paraffins influenced the performance of the birds in comparison to the 21 birds of the control (Group I). For evaluating the data, the experimental period of 8 weeks was further divided into the initial 4 weeks and the last 4 weeks to examine the CP effects with regard to the periods around the onset of laying and the laying peak, respectively. During the first four weeks of the experiment, the feed consumption of 107 g of the Control Group (Group I) did not significantly differ from the 109 g feed consumed *per* hen *per* day by the birds of the 100 mg/kg Group. Similar trends were observed after another four weeks, when birds of the Control Group consumed 115 g feed *per* day and hens of Group VI consumed nearly the same quantity of 118 g feed *per* hen *per* day ($p = 0.39$). Also, no significant differences were found in the two periods for egg weights ($p = 0.63$–0.78) and laying intensity ($p = 0.21$–0.51) for the hens with the 100 mg/kg additions in comparison to the control (Table 1). The daily egg mass and the feed conversion calculated for both individual periods from feed

Table 1. Performance of laying hens fed chlorinated paraffins (over the time of experiment indicated; start of experiment: 24 weeks of age; mean and SD of 21 animals each)

Group (mg chlorinated paraffins added *per* kg diet)	I (Control) (0)	VI (100)	ANOVA ($\alpha = 0.05$; $n = 21$) probability
Feed consumption (g/d × hen)			
Weeks 1–4	107 ± 10	109 ± 10	0.48
Weeks 5–8	115 ± 11	118 ± 10	0.39
Egg weight (g/egg)			
Week 4	57.4 ± 4.4	57.8 ± 4.0	0.78
Week 8	59.8 ± 5.2	60.5 ± 4.6	0.63
Laying intensity (egg × 100/hen × d)			
Weeks 1–4	94.0 ± 7.8	95.4 ± 5.4	0.51
Weeks 5–8	95.6 ± 6.0	97.6 ± 4.3	0.21
Daily egg mass (g/d × hen)			
Weeks 1–4	54.0 ± 5.8	55.2 ± 5.4	0.49
Weeks 5–8	57.1 ± 5.7	59.1 ± 5.3	0.25
Feed conversion (g/g egg mass)			
Weeks 1–4	1.99 ± 0.23	1.98 ± 0.16	0.87
Weeks 5–8	2.01 ± 0.14	1.99 ± 0.14	0.64

Table 2. Effects of chlorinated paraffins on mean live weights and relative organ weights of laying hens (week 32 of age, week 8 of experiment; mean and SD of nine animals)

Group (mg chlorinated paraffins added *per* kg diet)	I (Control) (0)	VI (100)	ANOVA ($\alpha = 0.05$; $n = 9$) probability
Live weight (g)[a)]	1488 ± 146	1521 ± 103	0.40
Relative organ weights (%)			
Liver	2.14 ± 0.18	2.14 ± 0.12	0.99
Kidneys	0.75 ± 0.10	0.74 ± 0.13	0.89
Pancreas	0.21 ± 0.014	0.18 ± 0.027	0.05
Thyroid gland	0.012 ± 0.007	0.017 ± 0.011	0.23
Heart	0.37 ± 0.05	0.34 ± 0.04	0.26
Gizzard	1.68 ± 0.24	1.56 ± 0.16	0.22
Spleen	0.096 ± 0.019	0.096 ± 0.017	0.99

a) Data are means of 21 animals.

consumption and daily egg mass were also similar in relation to the Control Group. Therefore, no evidence was found that the performance of hens might have been negatively influenced, even at very high CP additions used in the experiment.

3.2 Live weights, relative organ weights and animal health

The mean live weights of the hens ($n = 21$) of the Control Group and Group VI did not differ significantly at the end of the experiment (Table 2). This is in contrast to broilers where the birds of the 100 mg/kg Group had slightly reduced live weights with a worsened feed conversion ratio in relation to the control [15]. CP influence on live weights was also observed only at high concentrations with laboratory rodents orally dosed with 470–625 mg *per* kg body weight *per* day. A reduction in the egg fertility of wild ducks, and a slight decrease in egg-shell thickness, were observed at concentrations of 1000 mg CP *per* kg diet [18, 19].

As with broilers, the influence of CPs on the health and on the relative weights of most organs of laying hens at concentrations up to 100 mg/kg was minimal (Table 2). Just the absolute and the relative weights of the pancreas, related to the live weights of the hens of Group VI were lower in comparison to the Control Group. However, only the relative weights were significantly decreased. In contrast to broilers, the relative weights of the spleen of hens were not influenced at the highest dosing. Laboratory rodents had enlarged livers at dose levels of 100–470 mg CP *per* kg body weight *per* day when fed over a period of 14 days. After 90 days, the weights of kidneys and thyroid glands also increased [18, 19]. Slightly higher weights of thyroid glands (absolute and relative) were measured, but the effects were not significant. In addition, no signs of inflammation or other anomalies were found in the gastrointestinal tract by gross macroscopic inspection.

3.3 Carry over of chlorinated paraffins

The CP additions to the feed of 2/20/45/70 and 100 mg/kg were analytically recovered at 77 (62–84)%. Group I, with uncontaminated feed, served as control. The mean analysed content amounted to 0.14 ± 0.17 mg CP *per* kg feed. There was a strict linear relationship with determination values (R^2) >0.9 between the analysed CP content of the feed and the CP concentrations deposited into the tissues (Table 3). The highest CP concentrations in the diet (Group VI) corresponded to the highest values analysed in the tissues of animals of this group. This relation was found for all tissues and blood except for the bile fluid. Low CP contents in that fluid, nearly above the detection limit, rendered a high uncertainty, but even so no tendency of a concentration-dependent accumulation could be detected (Table 3). Saturation of the bile with CPs could be attained however, even at the low concentrations analysed. Therefore, the elimination of CPs *via* bile fluid – though to a minimal extent – cannot be excluded for laying hens. Very low CP concentrations were also analysed in bile fluid from broilers

Table 3. Effects of chlorinated paraffin-contaminated feed (x) on the concentration of chlorinated paraffins in tissues (y) (mg/kg wet weight) of laying hens after week 32 of age (tissue mix of nine animals; mix of eggs of nine animals)

Group (mg chlorinated paraffins *per* kg diet[a)]	I (0.14)	II (1.6)	III (12.3)	IV (37.9)	V (56)	VI (77)
	(mg chlorinated paraffins *per* kg wet weight)					
Abdominal fat ($y = 0.14x + 0.14$; $R^2 = 0.98$)	0.13 ± 0.05	0.74 ± 0.12	2.02 ± 0.12	4.25 ± 0.13	7.92 ± 0.65	11.1 ± 0.05
Yolk ($y = 0.08x - 0.14$; $R^2 = 0.97$)	0.03 ± 0.0	0.14 ± 0.0	1.00 ± 0.2	1.98 ± 0.09	4.43 ± 0.37	6.37 ± 0.73
Legs ($y = 0.008x + 0.085$; $R^2 = 0.98$)	0.05 ± 0.01	0.14 ± 0.04	0.20 ± 0.02	0.33 ± 0.13	0.54 ± 0.05	0.70 ± 0.05
Kidneys ($y = 0.012x + 0.052$; $R^2 = 0.99$)	0.05 ± 0.01	0.09 ± 0.0	0.14 ± 0.07	0.53 ± 0.04	0.71 ± 0.42	0.92 ± 0.16
Liver ($y = 0.012x + 0.014$; $R^2 = 0.98$)	0.02 ± 0.01	0.07 ± 0.03	0.17 ± 0.08	0.37 ± 0.01	0.73 ± 0.06	0.97 ± 0.1
Blood ($y = 0.008x + 0.048$; $R^2 = 0.99$)	0.04 ± 0.01	0.05 ± 0.0	0.20 ± 0.0	0.32 ± 0.01	0.53 ± 0.03	0.69 ± 0.11
Breast meat ($y = 0.004x + 0.002$; $R^2 = 0.64$)	0.03 ± 0.0	0.04 ± 0.01	0.07 ± 0.01	0.06 ± 0.06	0.10 ± 0.0	0.45 ± 0.54
Bile fluid	0.12	0.19	0.10	0.10 ± 0.02	0.19	0.14 ± 0.0
Droppings ($y = 0.22x + 0.25$; $R^2 = 0.99$)	0.11 ± 0.17	0.56 ± 0.08	3.03 ± 0.21	8.16 ± 0.85	13.4 ± 0.7	16.4 ± 0.18

a) Analysed content, $n = 3$, $n = 5$ (Group I Control).

[15]. The SCCPs (C10–C13) used in the experiment are characterised as lipophilic substances by the octanol:water partition coefficients (pK_{ow} of 4.5–7.5) and could consequently accumulate in fatty tissues. The fat content of the respective tissues, perfusion and turnover rates and other factors might influence the amount of CPs deposited. The highest concentrations were analysed thus in abdominal fat, yolk and liver of laying hens. No residues were found in the egg albumen containing low fat. The same dependence on the fat content was also found in tissues of broilers and other animals [5, 15]. With the higher fat content of leg meat in comparison to breast meat, correspondingly higher CP contents were analysed in the legs of hens when expressed on a wet weight basis. But when data are related to fat, the opposite holds true, which was due to the low fat level of breast meat. The data clearly showed that the CP accumulation in the animal tissues was dependent upon the administered dose. According to Wild and Jones [20], carry over or bioconcentration factors (BCFs) can be deduced from the linear regression coefficients as given in Table 3. These factors relate the CP concentrations of tissues to the CP concentrations of the feed and are expressed as percent. Approximately 14, 8 and 1% of the CP concentrations of the ingested feed can be expected in fat, yolk and liver (kidneys), respectively. When BCFs were expressed on a fat weight, the values of most tissues such as fat (97% fat), liver (9.7% fat), kidney (6.1% fat), leg (3.0% fat) and yolk (33% fat) were in a similar range (14/12/20/26/24%). This was also observed for other persistent and slowly metabolised compounds such as PCBs or dioxins [21, 22]. In these experiments the deviating BCF for liver was explained by specific binding to components other than fat.

Some limitations have to be considered in the interpretation of BCFs. Ideally these factors should be determined at equilibrium when the rates of ingestion and excretion are similar. However, the steady-state concentrations of persistent organics such as the CPs take a long time to attain, especially in body fat, due to low blood perfusion rates when contaminated feed is fed continuously. Therefore, feeding studies need to be carried out for a sufficiently long time. This was proven by following the increase in the CP concentration of yolk during the whole course of the experiment. When intake and elimination rates of CPs are at equilibrium, a plateau of concentration in the tissues is reached. This condition seemed to be fulfilled for yolk as the CP concentrations in yolk reached a steady-state concentration of approximately 6.3 mg/kg of continuous feeding in the diet with the highest CP supplements (Fig. 1). This asymptotic value was obtained by fitting the data to Eq. (2). From this value, a half-life of 8.3 days for the rise in CP concentration in the yolk could be calculated, and a nearly equilibrium concentration should be attained after 42 days by a continuous administration of the contaminated feed. By permanent exposure to the contaminant, a dynamic equilibrium (steady state) was reached between blood and the

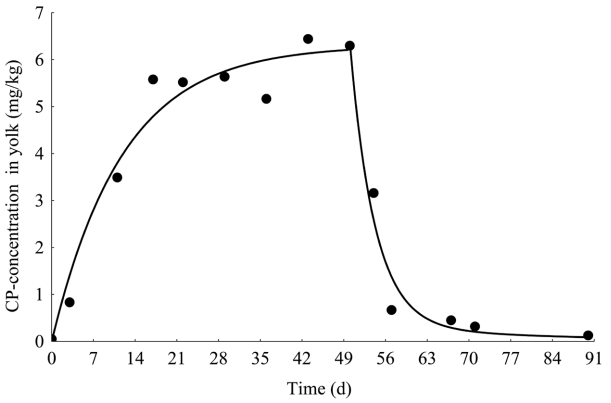

Figure 1. Concentration of CP in yolk after feeding 100 mg CP *per* kg (days 0-50) and withdrawing the contaminated feed (day >50).

different tissues after an initial phase of distribution. The level of the steady state is characteristic of tissue, and it is dependent on the lipophilicity of the contaminant or its isomers/congeners, which is characterised by the distribution coefficient of blood and tissue. The concentration level of the contaminant can also be influenced by the metabolic activity of the tissue. From the steady state in yolk, it may be concluded that the equilibrium CP concentrations in other tissues of similar or higher blood perfusion rates were also reached.

When an uncontaminated feed was supplied at the end of the experiment, the CP concentration in yolk dropped rapidly at the beginning and ended in slow phase (Fig. 1). The elimination of CPs was described by a biexponential time-dependent decrease with half-lifes of 2.9 and 20 days for rapid and slow elimination (Table 4), respectively. This may be explained by the rapid and slowly growing phase of oocytes during the egg formation in the hen's ovary. Only 5–7 of the follicles in the ovary are in the final phase of growth which takes 7–11 days. During this period, the yolk mass increases from 0.1 to about 18 g by taking up lipids and other nutrients from the blood [23]. As uncontaminated feed is given a progressive dilution of the contaminant already in the yolk forming oocytes might be assumed. After 7 days, the main amount (about 80%) of the contaminant included in follicles was eliminated by egg dropping.

All tissues showed a rapid and a slow phase of decrease in CP concentrations in the tissues after the supply of uncontaminated feed. However, this rapid phase was more distinct for yolk, blood and liver which made up 70–86%, and to a lesser degree for leg and kidney of about 20–40% of the total decrease in concentrations. The decline of CP concentrations of fat could mainly be described by the slow kinetic phase. In comparison to yolk, the elimination of CPs from the organs and tissues was much more rapid with half-lifes of about 42 min for blood and 4–10 min for liver, kidney and leg muscle. The terminal phase with half-liffes of

Table 4. Results of regressive evaluation of excretion curves of chlorinated paraffins in tissues and droppings ($n = 9$, pooled samples; tissue mix of three animals; droppings: from three or five animals; eggs: from six to ten animals each)

Model: $y = Ae^{-\alpha t} + Be^{-\beta t}$, y = mg chlorinated paraffins *per* kg wet weight; t = days (day); for yolk $t \geq 50$

	A (mg/kg)	α(1/day)	B (mg/kg)	β(1/day)	$t_{1/2}$ (α) (day)	$t_{1/2}$(β) (day)	R^2
Yolk[a)]	1 087 000	0.242	1.908	0.0342	2.86	20.3	0.97
Blood	6.505	23.96	0.0980	0.0421	0.029	16.5	0.99
Abdominal fat	−6.205	25.51	17.32	0.0407	0.027	17.0	0.65
Liver	0.769	219	0.200	0.0356	0.003	19.5	0.99
Kidneys	0.335	107	0.585	0.0253	0.006	27.4	0.77
Legs	0.138	124	0.562	0.0244	0.006	28.3	0.82
Droppings	−2.99	185	18.36	0.955	0.004	0.726	0.98

a) $n = 6$.

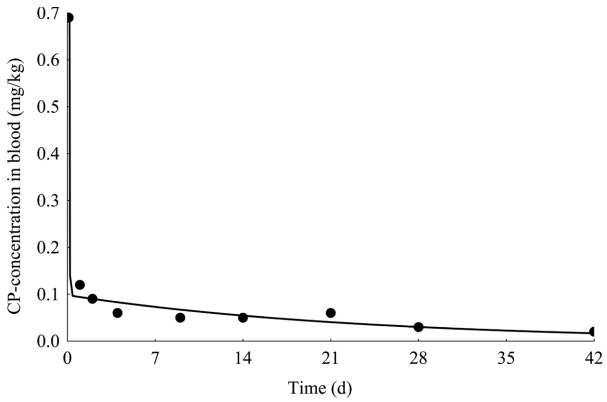

Figure 2. Elimination of CP in blood after withdrawing the contaminated feed.

20–30 days was nearly equal for all tissues and was assumed to be related to the equilibrium partitioning between blood and the slow perfused adipose tissues. The biphasic decrease has been observed for other persistent contaminants also, but for the PCBs only those congeners which are moderately stable to metabolism showed this biphasic kinetic behaviour [24]. Both metabolism and a rapid distribution could explain the most rapid decrease in liver whereas the rapid decline of the CP concentrations in blood (Fig. 2) may be caused by a rapid distribution into highly perfused tissues and excreta.

An input–output balance of chlorinated paraffins was roughly estimated for the animals of the 100 mg/kg group. The highest amount of about 30% of the ingested CP was excreted with the manure and urine. A relatively small proportion of approximately 1.5% was estimated for the yolk which was eliminated by egg dropping. Less than 1% remained in the body. The highest amounts were found in the skin, abdominal fat, ovary and meat, whose proportions of 16, 4.5 and 62% of the dressing and 60 g of the ovary weight were taken into account [25, 26]. Feet, head, feathers and ingesta were not considered. High CP concentrations, however, were analysed in feathers of ducks [5]. The excretion by exhalation was not considered, which is important for SCCPs of low chlorination. Most of the chlorinated paraffins are excreted, however, with faeces and urine [5]. SCCP stored in tissues which were not analysed, excretion by an unmeasured route, and errors in the estimates may account for the missing 65% in the mass balance.

As shown in this long-term study, laying hens stored CPs in abdominal fat, in the yolk and in their fat-containing tissues in as a function of the exposure time and in relation to the ingested amounts. Half-lifes of elimination of about 20 days precluded a rapid elimination from fatty tissues. However, as few survey data on the contamination level of feed and food are available, more studies are necessary to test whether the political restrictions are working, and to make an assessment of the human contamination to evaluate a possible risk to human health.

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4 References

[1] Zitko, V., Chlorinated Paraffins, in: Hutzinger, O. (Ed.), *The Handbook of Environmental Chemistry, 3A Anthropogenic Compounds*, Springer Verlag, Berlin 1980, pp. 149 – 156.

[2] IARC, Chlorinated Paraffins, in: International Agency for Research on Cancer (Ed.), *Some Flame Retardants and Textile Chemicals*, Lyon, 1990, IARC Monographs Vol. 48, pp. 55 – 72.

[3] Tomy, G. T., Fisk, A. T., Westmore, J. B., Muir, D. C. G., Environmental chemistry and toxicology of polychlorinated *n*-alkanes, *Rev. Environ. Contam. Toxicol.* 1998, *158*, 53 – 128.

[4] EU, Directive 2002/45/EC of 25 June 2002, *Off. J. Eur. Commun.*, L 177, 2002, 21 – 22.

[5] BUA, *Chlorparaffine*, *BUA-Stoffbericht 93*, VCH Verlagsgesellschaft, Weinheim, Germany 1992, ISBN 3-527-28602-0.

[6] OSPAR, *Short Chain Chlorinated Paraffins*, OSPAR Commission, London 2001, ISBN 0946 956 774.

[7] EU, *Opinion of the CSTEE on Short-chain chlorinated paraffins (SCCPs)*, Follow-up of the directive 2002/45/EC, Brussels, 17 December 2002.

[8] Campbell, I., McConnell, G., Chlorinated paraffins and the environment. 1. Environmental occurrence, *Environ. Sci. Technol.* 1980, *14*, 1209 – 1214.

[9] Nicholls, C. R., Allchin, C. R., Law, R. J., Levels of short and medium length polychlorinated *n*-alkanes in environmental samples from selected industrial areas in England and Wales, *Environ. Pollut.* 2001, *114*, 415 – 430.

[10] Stevens, J. L., Northcott, G. L., Stern, G. A., Tomy, G. T., Jones, K. C., PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated *n*-alkanes in U. K. sewage sludge: Survey results and implications, *Environ. Sci. Technol.* 2003, *37*, 462 – 467.

[11] Hartmann, E., Bischoff, W.-A., Kaupenjohann, M., *Untersuchung von Klärschlamm auf ausgewählte Schadstoffe und ihr Verhalten bei der landwirtschaftlichen Klärschlammverwertung*, Umweltbundesamt, Berlin 2004, Texte Umweltbundesamt 04/20, UBA-FB 000495.

[12] Jansson, B., Andersson, R., Asplund, L., Litzen, K., Nylund, K., *et al*., Chlorinated and brominated persistent organic compounds in biological samples from the environment, *Environ. Toxicol. Chem.* 1993, *12*, 1163 – 1174.

13] Schmid, P. P., Müller, M. M., Trace level detection of chlorinated paraffins in biological and environmental samples, using gas chromatography/mass spectrometry with negative-ion chemical ionization, *J. Assoc. Off. Anal. Chem.* 1985, *68*, 427 – 430.

[14] Kaiser, T., Schwarz, W., Frost, M., *Evaluierung des Gefährdungspotentials bisher wenig beachteter Stoffeinträge in Böden*, Umweltbundesamt, Berlin 1998, Texte Umweltbundesamt 98/60, UBA-FB 98 – 053.

[15] Ueberschär, K.-H., Matthes, S., Dose-response feeding study of chlorinated paraffins in broiler chickens: Effects on growth rate and tissue distribution, *Food Addit. Contam.*, 2004, *21*, 943 – 948.

[16] Lahaniatis, M. *Quantifizierung von kurz- und mittelkettigen Chlorparaffinrückständen in ausgesuchten fetthaltigen Lebensmitteln*, Dissertation, Fakultät für Ernährung, Landnutzung und Umwelt, Technische Universität München, 2001.

[17] Bergström, U., Jansson, B., Gas chromatographic method for the analysis of chlorinated paraffins in biological samples, *Organohalog. Comp.* 1998, *35*, 403 – 406.

[18] Serrone, D. M., Birtley, R. D. N., Weigand, W., Millischer, R., Toxicology of chlorinated paraffins, *Food Chem. Toxic.*, 1987, *25*, 553 – 562.

[19] Environmental Canada, *Priority Substances List Assessment Report – Chlorinated Paraffins*, Health and Welfare, Ottawa 1993, En 40 215/17 E, ISBN 0-662-20515-4.

[20] Wild, S. R., Jones, K. C., Organic chemicals entering agricultural soils in sewage sludges: Screening for their potential to transfer to crop plants and livestock, *Sci. Total Environ.* 1992, *119*, 85 – 119.

[21] Thomas, G. O., Sweetman, A. J., Jones, K. C., Input-output balance of polychlorinated biphenyls in a long-term study of lactating daily cows, *Environ. Sci. Technol.* 1999, *33*, 104 – 112.

[22] SCAN, *Dioxins in feed*, Opinion of the Scientific Committee on Animal Nutrition, EU-Com., Brussels 06/10/2000.

[23] Gerken, M., Krampitz, G., Petersen, J., Morphologischer Aufbau des Eies, in: Ternes, W., Acker, L., Scholtyssek, S. (Eds.), *Ei und Eiprodukte*, Paul Parey Verlag, Berlin, Hamburg 1994, pp. 50 – 81; 429 – 433.

[24] Tanabe, S., Nakagawa, Y., Tatsukawa, R., Absorption efficiency and biological half-life of individuals chlorobiphenyls in rats treated with Kanechlor products, *Agric. Biol. Chem.* 1981, *45*, 717 – 726.

[25] Pingel, H., Faustzahlen über Schlachtgeflügel, in: Petersen, J. (Ed.), *Jahrbuch für die Geflügelwirtschaft*, Eugen Ulmer Verlag, Stuttgart 2000, pp. 171 – 185.

[26] Gilbert, A. B., The Ovary, in: Bell, D. J., Freeman, B. M. (Eds.), *Physiology and Biochemistry of the Domestic Fowl*, Academic Press, London, New York 1971, Vol. 3, pp. 1163 – 1208.

