THE DISTRIBUTION AND METABOLISM OF LABELLED POLYCHLORINATED BIPHENYLS IN MICE AND QUAILS.

By

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MONS 086167

An extensive investigation has been started with the aim of studying and comparing the distribution and metabolism of several different polychlorobiphenyls varying with respect to number and position of chlorine atoms.

With this goal in view the synthesis of a number of ^{14}C-labelled compounds has been planned and started.

The distribution of these substances is being studied by whole body autoradiography in mice and birds and the biotransformation is simultaneously looked into.

So far a mixture of 2,3,4,2^1,4^1,6^1- and 2,4,6,3^1,4^1,5^1-hexachlorobiphenyl (I + II) and a mixture of 2,4,2^1,3^1- and 2,4,3^1,4^1-tetrachlorobiphenyl (III + IV) have been labelled by Wachtmeister. Labelled 2,4,2^1,4^1-tetrachlorobiphenyl (V) has been synthetised by Melvås.

Preliminary results from the investigations of tetrachlorobiphenyls in mice (V) and in quails (III + IV) are obtained and a review of the biological data so far available will be given.

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IDENTITY AND PURITY OF C^{14}(U)-LABELLED-2,4,2^1,4^1-TETRA-CHLOROBIPHENYL.

C^{14}(U)-labelled 1-chloro-2,4-dinitrobenzene with a specific activity of 25 µCi/mg were coupled according to Ullman (2) to 2,4,2^1,4^1-tetranitrobiphenyl. The nitro groups were then substituted with chlorine giving 2,4,2^1,4^1-tetrachlorobiphenyl (Figure 1 synthesis I). Melvås (3,4).

The purity was tested with gas-chromatography and thin layer chromatography. To confirm the structure we synthetized the same 2,4,2^1,4^1-tetrachlorobiphenyl with Ullman-coupling starting with unlabelled 1-iodo-2,4-dichlorobensene (Figure 1 synthesis II). Mass spectra were taken both on the labelled and unlabelled 2,4,2^1,4^1-tetrachlorobiphenyl.

Gas chromatography.

The gas chromatographic separations were made on a Varian 204 gas chromatograph.

Column: 1/8" m stainless steel.

Support: Chromosorb W AW DMCS 80/100 mesh.

Liquid phase: 2% SE-30.

Carrier gas: Nitrogen.

Flow rate: 20 ml/min.

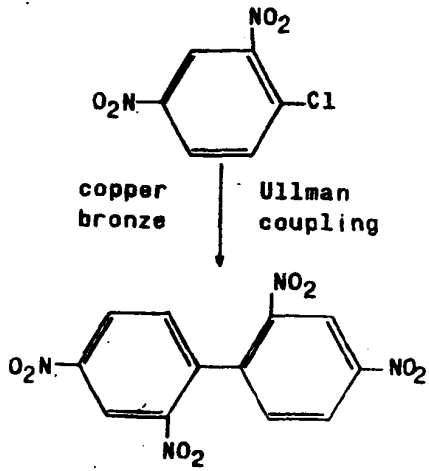
Detector: Flame ionisation.

Detector temperature: 270°C.

Injector temperature: 250°C.

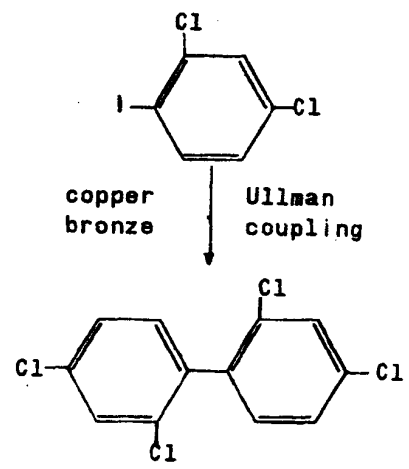
Synthesis I $C^{14}(U)$

1-chloro-2,4-dinitrobenzene



Synthesis II (unlabelled)

1-iodo-2,4-dichlorobenzene



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Flame ionization detector was preferred instead of electron capture detector in order to detect impurities others than those with high electron affinity. Both isothermal and programmed modes were used. When programmed from 80°C to 260°C only one single peak could be seen on the chromatogram. The tetrachlorobiphenyl was eluted at 179°C.

Mass spectrometry.

That lot of the labelled 2,4,2',4'-tetrachlorobiphenyl injected in the mice series were analyzed by mass spectrometry and so was the unlabelled 2,4,2',4'-tetrachlorobiphenyl synthetized from dichloro-iodo-benzene. Both mass spectra are identical within the error of the instrument.

Minispectra of 2,4,2',4'-tetrachlorobiphenyl

M/e	Starting materials: 1-chloro-2,4-dinitro-benzene C^{14}(U) (relative peak height in %)	Starting materials: 1-iodo-2,4-dichloro-benzene (unlabelled) (relative peak height in %)
119	17.2	13.5
150	32.4	20.2
181	14.0	13.1
220	68.6	67.4
222	44.7	48.3
290	76.0	79.5
291	15.7	16.7
292	100.0	100.0
293	16.2	16.7
294	46.6	49.0

Mass spectra of
2,4,2',4'-tetrachlorobiphenyl (C^{14} U)
starting from 2,4-dinitrochlorobenzene (C^{14} U)

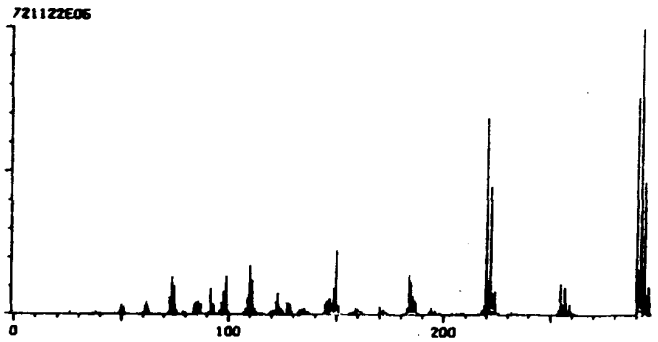

Figure 2

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Mass spectra of
2,4,2',4'-tetrachlorobiphenyl (unlabelled)
starting from 1-iodo-2,4-dichlorobenzene

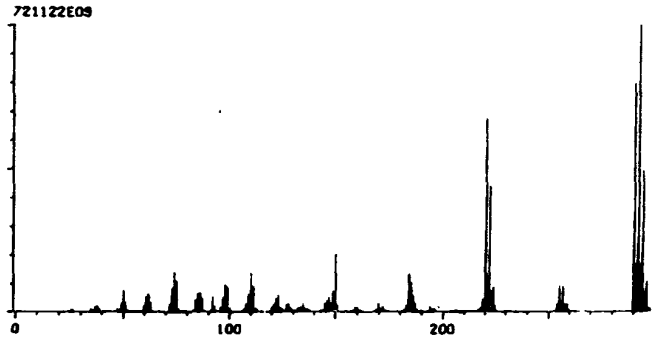

Figure 3

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Mass spectrometric conditions:

Ionisation voltage: 70 e V

Ionisation source temperature: 285°C

Separator temperature: 250°C

Column-liquid fase: OV 1

He flow: 20 ml/min.

The mass spectra were done on an LKB-GC-mass spectrometer at the crystallographic group, Institute of medical chemistry, University of Gothenburg.

Thin layer chromatography

Pre-coated 0.25 mm silicagel TLC-plates with fluorescent indicator F-254, 10 x 20 cm. (Merck 5729/0050) were used.

With heptane as a moving phase the radioactive compound got a RF-value of 46.

Autoradiography of the plate showed only one spot. No fluorescent spot could be detected at inspection in UV-light.

MONS 086174

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Ionisation source temperature: 285°C

Separator temperature: 250°C

Column-liquid fase: OV 1

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ANIMAL EXPERIMENTS

Metabolism.

The mice used in this series were of C57 black strain. Each mouse received in a tail vein an injection of 6 µCi 2,4,2^1,4^1-tetrachlorobiphenyl dissolved in 30 µl DMSO. The specific activity was 28 µCi/mg. Faeces and urine were collected in metabolism cages during 24 hour periods and stored in closed vials. In order to get a proximate figure of the relative rate of faecal excretion aliquots of each 24 hour faeces collection were placed under a GM-tube and counted under the same geometrical conditions.

The samples were measured three times, each time 200 or 800 secs and the average plotted (Figure 4). It can be seen that the amount excreted with the faeces from mice reaches a maximum a few days after the administration. This is in accordance with the autoradiographic data.

Of the collected faeces 500 mg samples were taken and extracted with diethylether (Merck zur Analyse) in a Soxhlet extractor with 8 ml sample capacity. The ether extract was evaporated to 2 ml. The concentrated ether solution was analysed with thin layer chromatography.

Following elution media were used: chloroform, chloroform-methanol (1:1), methanol, heptane-aceton (2:1), heptane and pentane.

Tetrachlorobiphenyl has an RF-value on silica gel with heptane as moving fase of 46, but the metabolites do not move at all. Comparative studies indicate a very close resemblance with hydroxybiphenyls.

The metabolites were indicated with autoradiography of the thin layer plates. The active compounds were scraped off and extracted for further analyses with gas chromatography and mass spectrometry.

2,4,2^1,4^1-tetrachlorobiphenyl and the mixture of 2,3,2^1,4^1- and 3,4,2^1,4^1-tetrachlorobiphenyl give both the same general excretion rates with a maximum at the first day and they also give metabolites with the same RF-value if chromatographed on silica gel with heptane: acetone (1:1). The mixture of hexachlorobiphenyl has not yet been analysed for metabolites but the faecce activity counting indicates a longer retention time than was the case for the tetrachlorobiphenyls.

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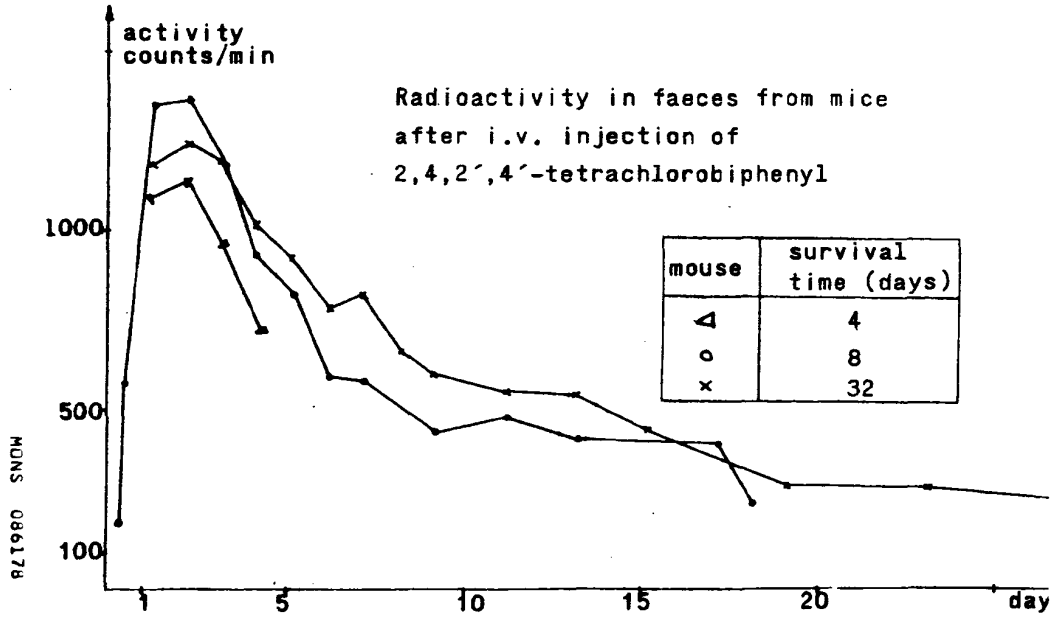
activity
counts/min
Radioactivity in faeces from mice
after i.v. injection of
2,4,2´,4´-tetrachlorobiphenyl
mouse
survival
time (days)
4
8
32
1000
500
100
1
5
10
15
20
day
MDNS 08617B

AUTORADIOGRAPHY (preliminary results)

A series of mice, strain C57 black, were injected with 6 µCi 2,1,2^{1},1^{1}-tetrachlorobiphenyl each in a tail vein. The substance corresponding to 0,12 mg was dissolved in 30 µl dimethylsulfoxide. The animals were killed after survival times from four minutes to 32 days. At the time of sacrifice the animals were anesthetized in chloroform and mounted in a gel of carboxymethyl cellulose and water. Then they were frozen to solid blocks in hexane cooled with solid carbon dioxide.

A series of male Chinese dwarf quails, Excalfactoria chinensis, weighing about 40 g each were injected with a preparation containing a mixture of 2,4,2^{1},3^{1}- and 2,4,3^{1},4^{1}-tetrachlorobiphenyl in a radial vein. The dose for each animal was 12 µCi corresponding to 0,14 mg. After survival times varying from four minutes to 30 days the birds were killed by anesthesia in carbon dioxide and plucked. Then they were frozen and mounted for sectioning as the mice.

Another series of laying Japanese quails, Coturnix coturnix japonica, weighing about 140 g each, were injected with 20 µCi of the same preparation and then anesthetized and frozen. Saggital sections attached to tape were taken at different levels of the body. The sections were dried at -15°C for a few days and then attached to X-ray film. Following exposure time up to eight months the autoradiograms were developed.

Distribution in mice (2,4,2',4' tetrachlorobiphenyl) and in quails (2,4,2',3' and 2,4,3',4'-tetrachlorobiphenyl).

Main findings in both species:

The most striking observation in the distribution pattern found in the mice was a very pronounced accumulation in the adrenal cortex and the corpora lutea of the ovaries. Short time after injection the concentration in these areas was considerably higher than in the fat but at later times the fat depots showed higher concentration than any other tissues.

High activity was seen in the salivary glands, Harder's gland and in the gastric mucosa in mice short time after injection.

The concentration was also rather high in the liver throughout the investigation period.

The concentration in the central nervous system was considerably lower and the level in the fetuses never approached the maternal.

In the quails the highest concentration in the body was found in the yolk 5 days after injection. As in the mice the fat and liver also were dominating.

In both the mice and the quails there was a rapid biliary excretion of radioactive material to the small intestine, where high activity was seen long time after injection.

Mouse (2,4,2',4'-tetrachlorobiphenyl)

4 min: Following intravenous administration the substance rapidly left the bloodstream. Four minutes after injection the activity in most tissues exceeded the blood level. The most dominating

accumulation at this survival time occurred in the adrenal cortex, and in the corpora lutea in the ovaries.

In the liver gastric mucosa and in the kidneys there was rather high activity.

Also in the salivary glands and in Harder's gland a pronounced accumulation was observed.

The concentration in the central nervous system was slightly higher than in the blood, but considerably lower than the myocardium. At this survival time the accumulation in body fat was below the liver level.

40 min: Forty minutes after administration the uptake in fat depots especially in the brown fat had increased compared to the liver. The activity in the gall bladder now exceeded that of the liver.

In the kidneys the concentration had decreased and now was of about the same magnitude as in the central nervous system. In the mammary glands the activity was slightly above the kidney level.

Labelled substance passed the placenta but the fetal concentration was considerably lower than the maternal. Within the fetuses the highest levels were found in the livers.

4 hrs: Four hours after injection the activity in the liver was beginning to decrease. The autoradiograms tended to be more dominated by the fat depots. In the liver the gall bladder contents showed activity far above that of the liver. Very heavy activity was seen in the intestinal contents apparently due to

biliary excretion. In the [illegible] nervous system the concentration had increased compared to the liver. The distribution pattern was becoming more distinct, especially in the cerebellum where a tendency to higher activity in the white matter could be seen.

24 hrs: Twentyfour hours after injection the fat depots were still more dominating. In the liver tissue a marked reduction in activity had occurred, while the concentration in the bile was at least of the same magnitude as in the subcutaneous fat.

4 and 8 days: Four and eight days after administration the level labelled substance retained in the fat depots was high compared to the liver. The activity in the central nervous system was very low at these survival times.

Quails.

The distribution in the cock quail following injection of 2,4,2^1,3^1 and 2,4,3^1,4^1- tetrachlorobiphenyl was as a whole in agreement with that of 2,4,2^1,4^1-tetrachlorobiphenyl in mice.

1 h: The activity in the blood did not decrease as fast as in the mice. One hour after injection the level active substance in the heart blood was about the same as in the myocardium and pectoral muscle.

The activity in the central nervous system was exceeding that of the blood. In the cerebellum the uptake gradually tended to be concentrated to the white matter.

A fairly high concentration of labelled substance was seen in the liver. In the kidneys the activity was about the same as in the central nervous system.

In the body fat the accumulation was high at this survival time. The most dominating concentration was, however, seen in the intestinal contents.

Also in the gizzard contents heavy concentration of labelled material could be seen, while the gizzard wall itself was almost lacking activity.

In the testes, the concentration was considerably lower than in the heart blood, but larger than in the gizzard wall.

Evidence for specific localization to endocrine organs has not been found.

4 hrs: Four hours after administration the fat accumulation was becoming more dominating in the autoradiograms together with the high level active substance excreted to the gizzard and intestines.

Autoradiograms from longer survival time were even more dominated by the retention in fat depots and excretion to the intestinal channel. Four days after administration the activity in the bile was far above that in the liver tissues.

Hen.

In the laying hen the distribution pattern was about the same as in the cock. The blood levels were, however, at all survival times higher, compared to the myocardium.

Labelled substance was heavily taken up and retained in the yolk of the follicle and egg. Five days after administration the activity in yolk exceeded all other tissues in the body. Active substance was poorly taken up by the albumen of the egg.

One hour after injection the activity in the oviduct was almost the same as in the kidney, but not so high as in the blood.

DISCUSSION

Polychlorinated biphenyls are established to be potent inducers of microsomal liver enzymes, increasing the metabolic rates of different steroid hormones (Risebrough et al. Lincer et al.). Different physiological disturbances following exposure to low doses polychlorinated biphenyls have been related to this phenomenon.

The most remarkable finding in this investigation was a very pronounced accumulation of labelled material in the adrenal cortex and corpora lutea in mice four minutes after injection of $2,4,2^{1},4^{1}$-tetrachlorobiphenyl. At this survival time the radioactivity in these sites exceeded all other tissues in the body. This rapid and selective uptake suggests a possible direct interference in the metabolism of steroid hormone secreting cells.

Following a daily dose of 70 mg technical PCB (kanechlor 400) per kg body weight for a month to rats, atrophy in the adrenal cortex was demonstrated (Inao et al.). The corticosterone producing capacity of the adrenals was significantly decreased. In cockerels fed 250 ppm Arochlor 1254 in the diet an antiandrogen effect, assessed by a decrease in testicular and comb growth, was observed (Platonow et al.).

Following injection of PCB (20 mg/kg) in mice prolonged estrous cycles were determined (Örberg et al.).

By means of the 18 hrs glycogen response in the immature rat uterus an estrogen effect was demonstrated following injection of different Arochlors containing up to 48% chlorine (Bitman et al.).

It is thus well established that low doses polychlorinated biphenyls reveals hormonal effects. It seems reasonable to assume that the disturbances in the steroid metabolism might be accomplished in two different ways. An increased hydroxylating capacity due to stimulation of microsomal enzymes in the liver gives more polar metabolites to be excreted. Besides, a direct toxic effect on the steroid hormone synthetizing organs, leading to an altered and/or decreased hormone production, is possible. The toxicology of polychlorinated biphenyls exhibit many similarities to that of DDT and its metabolites. DDT was shown to accumulate in the adrenal cortex and ovaries in mice (Bäckström et al.). Adrenal cortical atrophy and even necrosis was demonstrated in dogs following ingestion of DDD (Nelson et al.).

The corticosterone production in chicks was found to be inhibited by feeding subtoxic amounts of technical grade DDT. The degree of reduction was as a rule proportional to the dose given or to the concentration of DDT metabolites found in the adrenals (Srebocan et al.).

In the present investigation no uptake of labelled tetrachlorobiphenyl in endocrine organs has so far been observed in birds.

The distribution pattern of tetrachlorobiphenyl is characterized by its fat solubility. Within the first hour after injection the concentration in fat depots was very high. In spite of decreasing

activity in the fat tissues [illegible] from the longer survival times were dominated by these organs.

In the intestinal contents labelled substance was found already four minutes after injection in mice. Forty minutes after injection the radioactivity in the gall bladder was higher than in the liver tissue, indicating a rapid biliary excretion to the small intestine. Since at least the nonmetabolized polychlorinated biphenyls are almost completely absorbed from the intestines following peroral administration (Albro et al. it seems likely that a considerable enterohepatic circulation occurs.

In the laying hen labelled substance was heavily accumulated and retained in the yolk of the follicle and egg afer a single injection. It is obvious that small amounts of PCB this way might be transferred to the embryo and thus possiby cause disturbances in the development.

After administration of 50 mg Arochlor 1254 weekly to pheasants no significant difference in the hatchability, compared to a control group, could be seen. The number of eggs that were pipped but not hatched, however, increased significantly indicating a decreased viability of the embryo at the time of hatching (Dahlgren et al.).

Following injection of 10 mg Arochlor 1242 into embryonated White Leghorn eggs, only five percent of the eggs were hatched. Investigation of the dead fetuses revealed beak deformities, edema and growth retardation (McLaughlin et al.).

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