Acta vet. scand. 1978, *19*, 368—376.

From the Department of Toxicology, Biomedical Centre, University of Uppsala, Sweden.

TISSUE LOCALIZATION OF DDT AND TWO PCB ISOMERS (OCTA- AND TETRACHLOROBIPHENYL) IN LAYING QUAILS*

By

*Ingvar Brandt***, *Per-Gunnar Högman*, *Ywonne Larsson*** and *Sten Olsson*

BRANDT, I., P.-G. HÖGMAN, Y. LARSSON and S. OLSSON: *Tissue localization of DDT and two PCB isomers (octa- and tetrachlorobiphenyl) in laying quails.* Acta vet. scand. 1978, *19*, 368—376. — Whole-body autoradiography of DDT-^{14}C, 2,2′,4,5′-tetrachlorobiphenyl-^{14}C and 2,2′,3,3′,4,4′,5,6′-octachlorobiphenyl-^{14}C in laying quails indicated a strong deposition of radioactivity in the yolk of the growing follicles and the egg, confirming this to be a significant excretion route of lipophilic environmental pollutants in laying birds. A specific uptake of DDT and octachlorobiphenyl, but not of tetrachlorobiphenyl, was observed in the cortical cords of the adrenals. The octachlorobiphenyl was concentrated also in the ovarian stroma. Both DDT and the tetrachlorobiphenyl were excreted in the bile juice to the small intestine. No such excretion of octachlorobiphenyl was observed, indicating its rate of metabolization to be low. All compounds were accumulated and stored in the body fat. A previously described specific accumulation of 2,2′,4,5′-tetrachlorobiphenyl in the respiratory tract of mice was not observed in the quail.

DDT; polychlorinated biphenyls (PCB); autoradiography; quail; adrenal; ovary; yolk.

DDT and the polychlorinated biphenyls (PCB) belong to the most disseminated environmental pollutants. Due to their chemical stability and low polarity the compounds are transported and concentrated in the food webs, especially in aquatic environments. Consequently, different species at higher tropic levels are

* This investigation was supported by the Research Committee of the National Swedish Environment Protection Board.

** Present address: The Department of Pharmacology, Biomedical Centre, Faculty of Veterinary Medicine, The Swedish University of Agricultural Sciences, Uppsala, Sweden.

exposed to these environmental contaminants; this is particularly true for fish-eating birds and mammals. The Baltic seal population, e.g., is at present in a phase of rapid decline, mainly because of low breeding success. Although the seals show high body burdens of both DDT and polychlorinated biphenyls, it has been proposed that mainly the latter compounds are responsible for this failure in reproduction (*Helle et al.* 1976 a, b).

The toxic effects of DDT and different technical mixtures of PCB in birds have been thoroughly studied. Although species differences exist, the compounds are known to affect the egg production and hatchability. Embryonic growth and development have been impaired after prolonged exposure to both DDT and PCB (*Lillie et al.* 1971, 1974, 1975, *Briggs & Harris* 1973, *Britton et al.* 1974). However, while the DDT metabolite DDE produce egg shell thinning (*Haegele & Tucker* 1974), this effect has to our knowledge not been confirmed in experiments with PCB.

Previous autoradiographic studies of polychlorinated biphenyls in mice have generally established a close connection between their sites of toxic action and their localization in the body (*Brandt* 1975 a, b). Great differences, however, in the pattern of distribution of the different PCB isomers were observed (*Brandt* 1977).

In spite of the numerous investigations of DDT and PCB in avian species, the knowledge of their tissue distribution in birds is sparse (*Nagao & Hosoya* 1977). The aim of the present study has been to compare the autoradiographic localizations of DDT and two PCB isomers — 2,2′,4,5′-tetra- and 2,2′,3,3′,4,4′,5,6′-octachlorobiphenyl - – in laying quails with those previously obtained in experiments with mice (*Bäckström et al.* 1965, *Brandt* 1977).

MATERIALS AND METHODS

Uniformly labelled DDT-^{14}C (dichlorodiphenyltrichloroethane), spec. act. 30 mCi/mmol, was obtained from The Radiochemical Centre, Amersham. 2,2′,3,3′,4,4′,5,6′-octachlorobiphenyl-^{14}C and 2,2′,4,5′-tetrachlorobiphenyl-^{14}C, both with the spec. act. 25 mCi/mmol, were provided by Dr. C. A. Wachtmeister and his co-workers at Wallenberg Laboratory, University of Stockholm, Stockholm. The octachlorobiphenyl-^{14}C was uniformly labelled in the 2,3,4,6-substituted ring and the tetrachlorobiphenyl-

^{14}C in the 2,4-substituted ring (*Sundström* 1973, 1974, *Bergman & Wachtmeister* 1977). The purity of the DDT-^{14}C-preparation was 99 % (TLC-radiochromatogram scanning). Both the PCB isomers were radiochemically pure (GLC, TLC-autoradiography).

For the experiments with DDT-^{14}C and the tetrachlorobiphenyl-^{14}C, laying Japanese quails (Coturnix coturnix japonica) — body weight about 150 g each — were used. In the experiment with the octachlorobiphenyl-^{14}C, laying Chinese quails (Excalfactoria chinensis) — body weight about 50 g each — were utilized.

Autoradiography

The labelled compounds were dissolved in dimethyl sulfoxide (DMSO). Two series of Japanese quails were intravenously injected with 40 µl of the solutions of DDT-^{14}C and 2,2′,4,5′-tetrachlorobiphenyl-^{14}C, respectively; each bird receiving 50 µCi DDT-^{14}C (corresponding to 1.67 µmol) or 10 µCi tetrachlorobiphenyl-^{14}C (corresponding to 0.40 µmol). The birds of a third series of Chinese quails each received 10 µCi (0.40 µmol) of the octachlorobiphenyl-^{14}C preparation in 20 µl DMSO orally via a stomach tube. Special care was taken to ensure that the tube passed the crop and that the labelled solution was deposited below the gizzard. The injected birds were killed in an atmosphere of carbon dioxide at the different post injection times scheduled in Table 1.

The feathers were thoroughly plucked off, whereupon the wings and the legs were removed. The birds were then mounted in an aqueous gel of carboxymethyl cellulose (CMC) and frozen in a bath of hexane and solid carbon dioxide (—70°C). According to the Ullberg technique (*Ullberg* 1954, 1977) sagittal whole body sections (20 and 60 µm) from different levels of the body were then collected onto tape (3 M, No. 810) in a PMV-cryostat microtome (Palmstiernas mekaniska verkstad, Stockholm). After freeze-drying, the sections were gently apposed to Roentgen film (Industrex® Kodak) for autoradiographic exposure. In order to prevent artefacts due to the melting and diffusion of fat in the dehydrated sections, all manipulations with the biological material were carried out at —20°C (*Appelgren* 1967). After exposure times ranging from 14 days to 6 months the film was separated from the sections, developed, fixed and rinsed. Suitable sections were stained in hematoxylin-eosin and mounted under cover glasses for microscopic examination.

Table 1. Injection regimen of the labelled compounds.

	Time between administration and freezing			
	4 hrs.	24 hrs.	4 days	10 days
DDT-^{14}C	1	1	1	
Tetrachlorobiphenyl-^{14}C	1	1	1	1
Octachlorobiphenyl-^{14}C	1	1	1	

RESULTS

DDT-^{14}C

The distribution pattern was dominated by a strong uptake of radioactivity in the yolk of the growing follicles and the egg. The radioactivity here was confined to the peripheral layers corresponding to the yolk formed between the time of injection and sacrifice (Figs. 1—2). A strong and increasing uptake of labelled substance was observed also in the body fat. Although less than in the yolk and fat tissue, the concentration in the liver was high and completely even. Also in the sites of the adrenal corresponding to the cortical cells, a concentration of radioactivity slightly below that of the liver was observed throughout the investigative period. The concentration in the circulating blood was moderate and roughly equal to that of the kidney, lung and brain. There was a biliary excretion of labelled material to the small intestine, but the concentration in the intestinal tract decreased considerably with time. In the cecae, however, a strong retention of radioactivity was observed still 4 days after injection.

2,2',3,3',4,4',5,6'-octachlorobiphenyl-^{14}C

Four hrs. up to 4 days after administration the most dominating site of localization was the yolk of the growing ovarian follicles and the egg (Fig. 3). The same zonal pattern of deposition of the radioactivity was observed as in the DDT-injected quails. The concentration in the body fat appeared to increase with increasing post injection time, the fat tissues and the yolk being the most dominating reservoirs of PCB after 4 days. Also in the liver a strong and completely evenly distributed uptake was observed, with no site of localization within the liver lobule corresponding to that previously observed in mice (*Brandt* 1977). As indicated by the absence of radioactivity in the small intestine

24 hrs. and 4 days after peroral administration, the metabolism and rate of biliary excretion of the octachlorobiphenyl were low. The concentration in the circulating blood remained comparatively high throughout the whole investigation (Fig. 3). In the central nervous system the uptake was also fairly high, with a peak concentration after 24 hrs. (Fig. 3). In the sites of the adrenal corresponding to the cortical cords, a marked accumulation was observed at all survival times, the activity being about equal to that of the hepatic tissue (Fig. 4). Also in the ovarian stroma a selective localization of octachlorobiphenyl occurred (Fig. 4).

2,2',4,5'-tetrachlorobiphenyl-^{14}C

The distribution pattern of the tetrachlorobiphenyl-^{14}C about equalled that observed for DDT-^{14}C (Fig. 1). There was, however, a stronger labelling of the intestinal contents in the quails injected with the tetrachlorobiphenyl-^{14}C, indicating a more rapid metabolism and excretion of the latter compound. The tetrachlorobiphenyl-^{14}C was evenly distributed throughout the lung tissue with no site of accumulation in the respiratory pathways corresponding to that previously found for this PCB isomer in mice (*Brandt* 1977). By far, the highest concentration in the body was observed in the yolk 1 day after injection. In the quail killed 10 days after injection the radioactivity in the body fat and the yolk was about equal, indicating a distribution equilibrium to be reached. The concentration in the adrenals was consistently low compared to the DDT-^{14}C and octachlorobiphenyl-^{14}C injected birds.

DISCUSSION

The most dominating feature in the distribution patterns of the compounds studied was a pronounced uptake of radioactivity in the follicular and egg yolk. The incorporation of radioactivity here essentially followed the deposition of lipids in the growing follicles, as was indicated by a zonal distribution in the yolk. These zones became wider with increasing survival time showing an almost complete uptake throughout the yolk after 12 days. As indicated by the variation in the density of silver grains — in the parts of the autoradiograms representing yolk formed at different periods after injection — the incorporation of radioactivity seemed to follow the diurnal rhythm of the yolk formation.

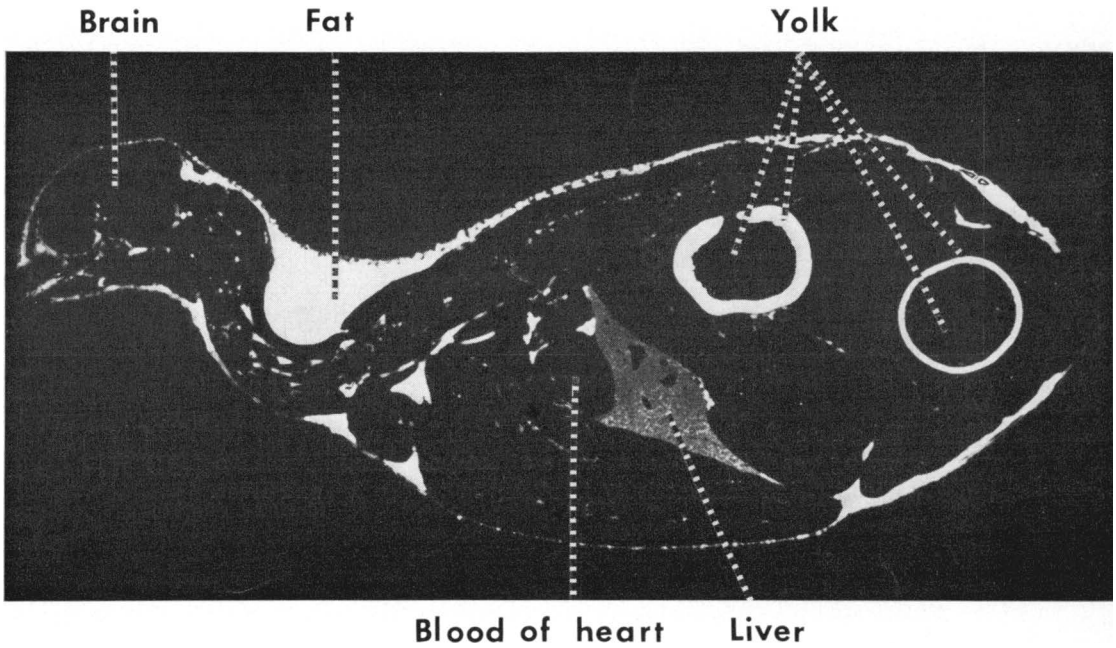

Figure 1. Autoradiogram of a laying quail 24 hrs. after i.v. injection of DDT-^{14}C. Except for a strong uptake of radioactivity in the outer layers of the yolk, the body fat and liver, the concentration in the visible tissues is fairly low.

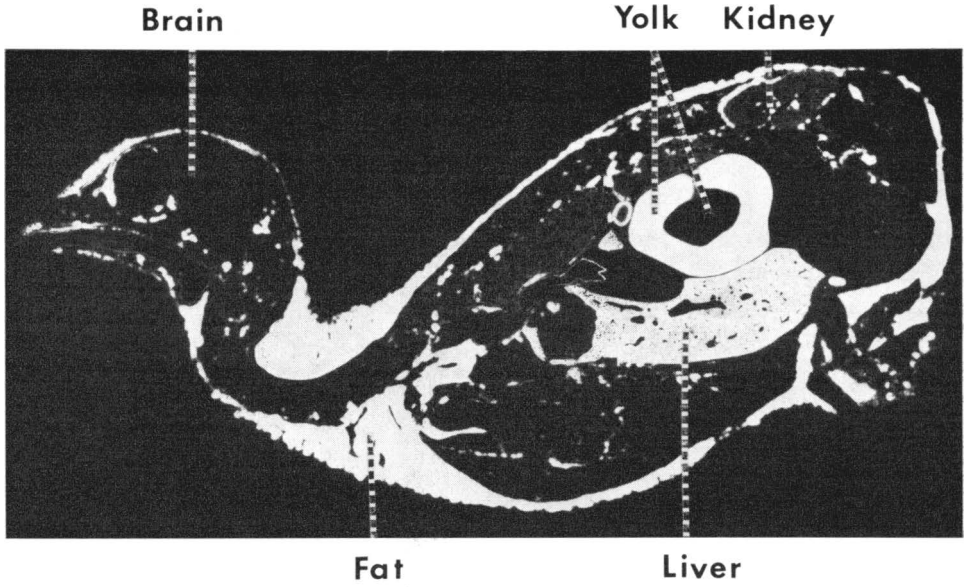

Figure 2. Distribution of DDT-^{14}C 4 days after i.v. injection in a laying quail. The labelled zone of the yolk has increased compared to Fig. 1. High radioactivity is observed in the fat and liver.

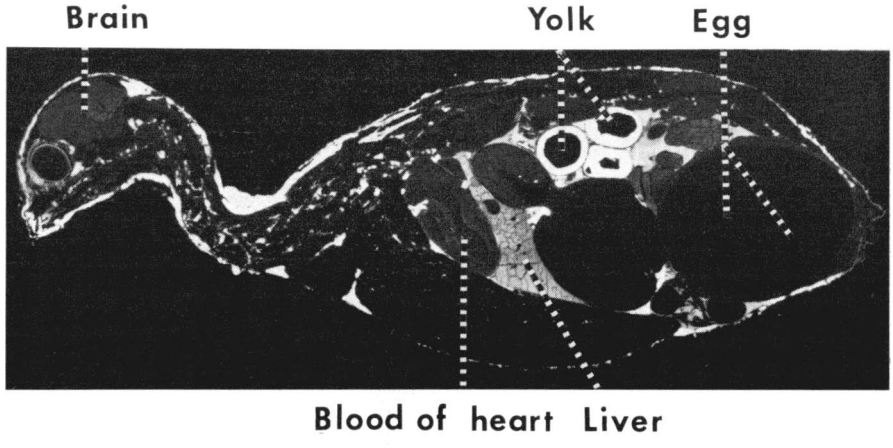

Figure 3. Autoradiogram of a laying quail 24 hrs. after p.o. administration of octachlorobiphenyl-^{14}C. Note the marked deposition of PCB in the yolk of the growing follicles and in the body fat (neck region). The radioactivity in the visceral organs is comparatively high compared to the DDT-^{14}C injected birds.

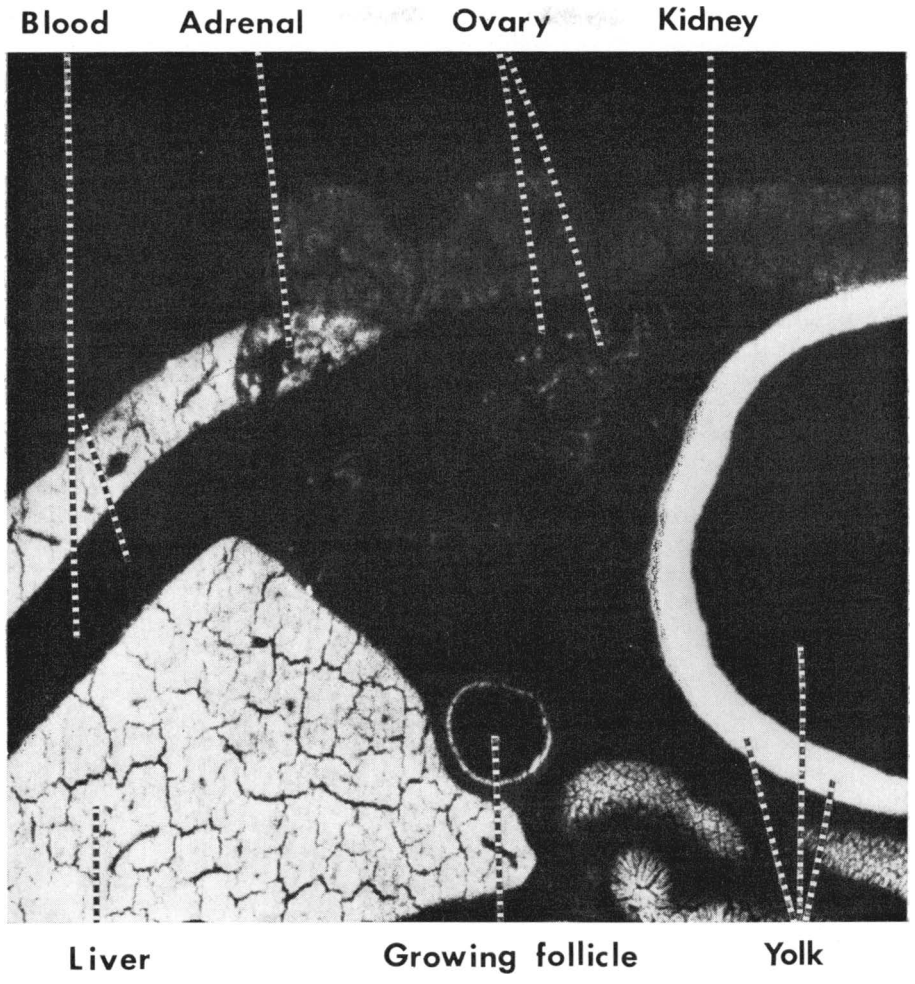

Figure 4. Detail of an autoradiogram showing the uptake of octachlorobiphenyl-^{14}C in the cortical cell regions of the adrenal in a laying quail 4 hrs. after p.o. administration. A certain concentration of PCB is seen also in the ovarian stroma. Note the high radioactivity in the yolk of a growing and a mature follicle.

The uptake in the yolk can in part be a process of diffusion from the vessels in the follicular surface. There may, however, also be an uptake of PCB or DDT in circulating yolk lipoproteins of the blood and a subsequent incorporation with the follicular growth. In fact the blood concentrations in laying quails were considerably higher than those observed in mice, this was particularly true for the octachlorobiphenyl.

Although metabolites of DDT and PCB can be incorporated, it seems likely that the bulk of radioactivity in the yolk represents the unmetabolized compounds (*Tumasonis et al.* 1973). The deposition in yolk appears to be a general excretion mechanism of lipophilic environmental pollutants in birds. At least in continually laying species this may be a significant route of elimination of chlorinated hydrocarbons, which are more or less resistant to metabolic degradation (*Tumasonis et al., Ivie et al.* 1974). The route of excretion also implies that the birds will be exposed to PCB and DDT already during their embryonic and fetal life. This seems to be a factor behind the decreased hatchability observed under experimental and natural conditions (*Tumasonis et al.*).

The selective uptake of the octachlorobiphenyl-^{14}C and DDT-^{14}C in the cortical cords of the adrenals equals that previously observed in the adrenal cortex of mice (*Bäckström et al.* 1965, *Brandt* 1977). The autoradiograms also demonstrated a marked uptake of octachlorobiphenyl-^{14}C in the ovarian stroma. This octachlorobiphenyl showed a similar affinity for the mouse ovarian stroma, thus contrasting to the specific accumulation of some hexachlorobiphenyl isomers in the corpora lutea of pregnant mice (*Brandt* 1977).

The effects of PCB and DDT on hormonal homeostasis in mammals and birds are well documented. In addition to observed increased rates of hepatic breakdown of the endogenous steroid hormones (*Lincer & Peakall* 1970, *Nowicki & Norman* 1972), these effects may also depend on a direct action of certain PCB isomers and DDT on the function of the steroid hormone producing tissues (*Srebočan et al.* 1971, *Jonsson Jr. et al.* 1975/76, *Brandt* 1977).

Unlike the situation in the mouse, there was no accumulation of 2,2′,4,5′-tetrachlorobiphenyl-^{14}C in the respiratory pathways of the Japanese quail. In the former species, PCB isomers fulfilling certain structural criteria have been found to be heavily

accumulated in the mucosa of the bronchi, trachea and larynx (*Brandt et al.* 1976, *Brandt* 1977). We have recently shown that the bronchial-seeking PCB isomers are deposited in the respiratory tract as methylsulfonyl derivatives (*Bergman et al.* in press), a new type of PCB metabolites recently identified in seals and mice (*Jensen & Jansson* 1976, *Mio et al.* 1976). The absence of this bronchial accumulation in the Japanese quail may possibly depend on a different metabolism, but can also reflect a functional difference in the respiratory tract. Notably the methylsulfonyl metabolites of PCB have been identified also in birds, but the current knowledge indicates that there are qualitative differences in the methylsulfonyl spectrum of different avian species (*Jansson et al.* in preparation).

ACKNOWLEDGEMENTS

We are indebted to Professor Carl Axel Wachtmeister and his co-workers Drs. Åke Bergman and Göran Sundström for the synthesis of the labelled polychlorobiphenyls. Miss Raili Pensas and Mr. Hans Sundberg are acknowledged for skilful technical assistance.

REFERENCES

Appelgren, L.-E.: Sites of steroid hormone formation. Autoradiographic studies using labelled precursors. Acta physiol. scand. 1967, Suppl. 301, 1—108.

Bäckström, J., E. Hansson & S. Ullberg: Distribution of C^{14}-DDT and C^{14}-dieldrin in pregnant mice determined by whole-body autoradiography. Toxicol. appl. Pharmacol. 1965, 7, 90—96.

Bergman, Å. & C. A. Wachtmeister: Synthesis of (^{14}C)biphenyl and some chlorinated (^{14}C)biphenyls containing 4-chloro-, 2,4-dichloro- and 2,3,6-trichlorophenyl nuclei from the corresponding labelled anilines. Chemosphere 1977, *11*, 759—767.

Bergman, Å., Brandt, I. & B. Jansson: Accumulation of methylsulfonyl derivatives of some bronchial-seeking polychlorinated biphenyls in the respiratory tract of mice. Toxicol. appl. Pharmacol. In press.

Brandt, I.: The distribution of 2,2′,3,4,4′,6′- and 2,3′,4,4′,5′,6-hexachlorobiphenyl in mice studied by whole-body autoradiography. Toxicology 1975a, *4*, 275—287.

Brandt, I.: Distribution of 2,2′,4,4′,5,5′-hexachlorobiphenyl in mice and Chinese hamsters. Arch. Toxicol. 1975b, *34*, 111—119.

Brandt, I.: Tissue localization of polychlorinated biphenyls. Chemical structure related to pattern of distribution. Acta pharmacol. (Kbh.) 1977, *40*, Suppl. 2, 1—108.

Brandt, I., A. Bergman & C. A. Wachtmeister: Distribution of polychlorinated biphenyls: Structural requirements for accumulation in the mouse bronchial mucosa. Experientia (Basel) 1976, *32*, 497—498.

Briggs, D. M. & J. R. Harris: Polychlorinated biphenyls influence on hatchability. Poultry Sci. 1973, *52*, 1291—1294.

Britton, W. M., M. B. Palmer & B. Howarth, Jr.: Fertility, hatchability and progeny performance of laying hens fed high levels of DDT in the diet. Poultry Sci. 1974, *53*, 1053—1055.

Haegele, M. A. & R. K. Tucker: Effects of 15 common environmental pollutants on eggshell thickness in mallards and coturnix. Bull. environm. Contam. Toxicol. 1974, *11*, 98—102.

Helle, E., M. Olsson & S. Jensen: DDT and PCB levels and reproduction in ringed seal from the Bothnian Bay. Ambio 1976a, *5*, 188—189.

Helle, E., M. Olsson & S. Jensen: PCB levels correlated with pathological changes in seal uteri. Ambio 1976b, *5*, 261—263.

Ivie, G. W., H. W. Dorough & H. E. Bryant: Fate of Mirex-^{14}C in Japanese quail. Bull. environm. Contam. Toxicol. 1974, *11*, 129—135.

Jensen, S. & B. Jansson: Anthropogenic substances in seal from the Baltic: methylsulfone metabolites of PCB and DDE. Ambio 1976, *5*, 257—260.

Jonsson Jr., H. T., J. E. Keil, R. C. Gaddy, C. B. Loadholt, G. R. Hennigar & E. M. Walker Jr.: Prolonged ingestion of commercial DDT and PCB; effects on progesterone levels and reproduction in the mature female rat. Arch. environm. Contam. Toxicol. 1975/1976, *3*, 479—490.

Lillie, R. J., C. A. Denton, H. C. Cecil, J. Bitman & G. F. Fries: Effect of p,p'-DDT, o,p'-DDT and p,p'-DDE on the reproductive performance of caged white leghorns. Poultry Sci. 1971, *50*, 1597—1598.

Lillie, R. J., H. C. Cecil, J. Bitman & G. F. Fries: Differences in response of caged white leghorn layers to various polychlorinated biphenyls (PCBs) in the diet. Poultry Sci. 1974, *53*, 726—732.

Lillie, R. J., H. C. Cecil, J. Bitman, G. F. Fries & J. Verrett: Toxicity of certain polychlorinated and polybrominated biphenyls on reproductive efficiency of caged chickens. Poultry Sci. 1975, *54*, 1550—1555.

Lincer, J. L. & D. B. Peakall: Metabolic effects of polychlorinated biphenyls in the American kestrel. Nature (Lond.) 1970, *228*, 783—784.

Mio, T., K. Sumino & T. Mizutani: Sulfur-containing metabolites of 2,5,2',5'-tetrachlorobiphenyl — a major component of commercial PCB's. Chem. pharm. Bull. 1976, *24*, 1958—1960.

Nagao, S. & H. Hosoya: Distribution of ^{14}C-labelled PCB and BHC in the Japanese quail by whole-body autoradiography. Acta pharmacol. (Kbh.) 1977, *41*, Suppl. 1, 146—147.

Nowicki, H. G. & A. W. Norman: Enchanced hepatic metabolism of testosterone, 4-androstene-3, 17-dione, and estradiol-17β in chickens pretreated with DDT or PCB. Steroids 1972, *19*, 85—99.

Srebočan, V., J. P. Gotal, V. Adamović, B. Sokić & M. Delak: Effect of technical grade DDT and p,p′-DDT on adrenocortical function in chicks. Poultry Sci. 1971, *50*, 1271—1278.

Sundström, G.: Polychlorinated biphenyls. IV. Synthesis of 2,2′,3,3′, 4,4′,5,6′-octachlorobiphenyl-^{14}C from 3-nitro-2,2′,3′,4,4′,5,6′-hepachlorobiphenyl-^{14}-C and tetrachloromethane. Acta chem. scand. 1973, *27*, 1109—1111.

Sundström, G.: Polychlorinated biphenyls. V. Synthesis of ^{14}C-labelled 2,2′,4,4′,5,5′-hexachlorobiphenyl, a major component in PCB-mixtures. Bull. environm. Contam. Toxicol. 1974, *11*, 39—44.

Tumasonis, C. F., B. Bush & F. D. Baker: PCB levels in egg yolks associated with embryonic mortality and deformity of hatched chicks. Arch. environm. Contam. Toxicol. 1973, *1*, 312—324.

Ullberg, S.: Studies on the distribution and fate of S^{35}-labellel benzylpenicillin in the body. Acta radiol. (Stockh.) 1954, Suppl. 118, 1—110.

Ullberg, S.: The technique of whole-body autoradiography. Cryosectioning of large specimens. Sci. Tools 1977, Special issue, 2—29.

SAMMANFATTNING

Vävnadslokalisation av DDT och två PCB-isomerer (okta- och tetraklorbifenyl) i värpande vaktelhöna.

Distributionen av DDT-^{14}C, 2,2′4,5′-tetraklorbifenyl-^{14}C och 2,2′, 3,3′,4,4′,5,6′-oktaklorbifenyl-^{14}C studerades med helkroppsautoradiografi. Samtliga substanser koncentrerades i äggets och de växande folliklarnas gula, vilket visar att detta är en betydande utsöndringsväg för lipofila miljögifter hos värpande fågel. Vidare sågs en uttalad ackumulation av DDT och oktaklorbifenyl i binjurens kortikalsträngar. Oktaklorbifenyl koncentrerades dessutom i ovariets stroma. Både DDT och tetraklorbifenyl utsöndrades med galla till tarmen. Oktaklorbifenyl koncentrerades däremot ej i galla och tarm, vilket antyder att denna PCB metaboliseras långsamt. Samtliga substanser ackumulerades och retinerades i kroppsfett. Tetraklorbifenyl, som tidigare visats ha specifik affinitet till bronkialslemhinnan hos mus, anrikades inte i respirationsvägarna hos vaktel.

(Received February 11, 1978).

Reprints may be requested from: Ingvar Brandt, the Department of Pharmacology, Biomedical Centre, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Box 573, S-751 23 Uppsala, Sweden.