into two 0.5 ml microcuvettes, bubbling one of them with CO and determining the difference spectrum on a recording spectrophotometer. The ethyl isocyanide difference spectrum was measured similarly. The cytochrome b5 difference spectrum was obtained similarly using 1 mM NADH or NADPH to produce the difference. The protein level was twofold greater in the females. The females also had higher cytochrome P-450 levels on a per-fly basis, but males had higher P-450 content on a microsomal protein basis. The substrate specificity ratio showed a marked specificity of the Fe and R-Baygon resistant microsomes for DDT and PNA (*p*-nitroanisole) metabolism, respectively. The cytochrome b5 content did not differ with sex or strain on a per-fly basis, but on a microsomal protein basis, the males had the higher content. The b5 level was increased slightly in both sexes of the Orlando DDT-resistant strain and cytochrome P-450 was increased more in the females than the males by nontoxic doses of dieldrin. Piperonyl butoxide applied to the thorax resulted in increased b5 level when NADPH was used for reduction and a decrease in P-450 determined with ethyl isocyanide. Piperonyl butoxide added to the microsomal suspensions gave the same results. The results indicated that the cytochrome b5 increase is neither sex- nor strain-dependent, while the difference in cytochrome P-450 is both sex-and strain-dependent.

71-0802. Sutherland, D. J.; Sierwierski, M.; Marei, A. H.; Helrich, H. (Dept. of Entomology and Economic Zoology, Rutgers–The State U., New Brunswick, N. J. 08903). The effect on mosquitoes of sublethal exposure to insecticides. II. DDT metabolism. *Mosquito News 30(1): 8-11; 1970.* (14 references)

The uptake and metabolism of DDT in mosquito larvae (*Aedes aegypti*) exposed to the insecticide were investigated. Approximately 600 mosquito larvae that had hatched about 1 hr earlier were placed in enameled pans containing water treated with 0.001 ppm DDT (in ethanol). About 0.8 g of ground Purina Laboratory Food was added to the water daily. The pans were covered loosely with aluminum foil and maintained at 30.5 C. On days 3 and 5, respectively, total larval and pupal populations of individual pans were counted, recovered by filtration, rinsed with fresh water and homogenized in 5 to 10 ml of acetone. Adult populations from each pan were trapped, counted and homogenized in acetone. Homogenates were filtered through a sintered glass funnel. The acetone-water solutions remaining after the acetone washings were extracted with hexane, concentrated and passed through Florisil. The sample was eluted with ethyl ether in hexane and then concentrated before being injected into a Micro Tek MT 200 gas chromatograph equipped with a Ni-63 electron-capture detector. The methods used for the analyses were those of Siewierski and Helrich (1967). Recovery values for DDE, DDD and DDT were 90.1, 84.8 and 81.7%, respectively. DDT was adsorbed during the early larval stages. The findings showed that DDT was metabolized slowly during the pupal and early adult stages, while DDD was rapidly metabolized by the mosquito and/or microorganisms. The amounts of DDD found in the adult mosquito were negligible. The findings suggest that immature mosquitoes containing sublethal quantities of DDT which emerge as adults may serve as an efficient dispersal agent of this insecticide and its metabolites from aqueous to terrestrial environments.

71-0803. Godfraind, J. M.; Krnjevic, K.; Pumain, R. (Dept. of Res. in Anaesthesia, McGill U., Montreal, Quebec, Canada). Unexpected features of the action of dinitrophenol on cortical neurones. *Nature 228(5271): 562-4; 1970.* (28 references)

The effect of 2,4-dinitrophenol (DNP) on cortical neurons of cats was investigated. Test animals were anesthetized with a mixture of nitrous oxide and methoxyflurane. Succinylcholine was infused i.v. to reduce pulsation of the brain. The techniques described earlier by Krnjevic and Schwartz (1967) and Dreifuss *et al.* (1969) were used to record the membrane potential and resistance as well as extracellular microiontophoresis. The release of DNP from a 0.1 M sodium salt solution was effected at pH 9 by an inward current (microelectrode relatively negative). Immediate estimates of the membrane resistance and the reversal levels of inhibitory synaptic potentials (IPSPs) were obtained in some experiments in which a Line 8 computer was used on-line to apply pulses, sample membrane potentials and derive voltage-current lines (Kelly, 1969). Electrical excitability was markedly decreased by the action of DNP. This decrease was associated with a tendency to hyperpolarization and a lower resistance of the membrane. The possible explanations for these observations are discussed. Intracellular recordings showing the effect of DNP and acetylcholine in the pretuciate neuron and recordings showing IPSPs in the neuron by surface shocks (0.1 ms, 12 V, at 3 Hz) are illustrated and detailed.

71-0804. Lincer, J. L.; Peakall, D. B. (Langmuir Lab., Sect. of Ecology and Systematics, Cornell U., Ithaca, N. Y. 14850). Metabolic effects of polychlorinated biphenyls in the American kestrel. *Nature 228(5273): 783-4; 1970.* (14 references)

The metabolic effects of Aroclor 1254 (54% chlorine) and Aroclor 1262 (62% chlorine) in the American kestrel were investigated. American kestrels were trapped and acclimatized for 1 week. Male and female birds were paired, acclimatized for another week and then transferred one pair/cage to burlap-covered wire cages and fed once a day for 5 months with 1-day-old dead cockerels that had been injected in the breast region with 0.5 or 5.0 ppm Aroclor 1254 or 1262 in sesame oil. Kestrels which were fed on cockerels injected with sesame oil only served as controls. Kestrels were sacrificed at the end of the experiment and their livers were excised. The microsomal fraction was prepared and incubated as detailed by Conney and Klutch (1963). Radioactive estradiol and its metabolites were extracted with dichloromethane and separated by paper chromatography (Kuntzman *et al.*, 1966) as modified by Peakall and Risebrough *et al.* (1967 and 1969). Histological examinations were carried out on the livers of kestrels which were fed a diet containing 5 ppm Aroclor 1254 for 14 days. Tissue sections were fixed in Carnoy's fluid and treated with deoxyribonuclease to remove DNA which could interfere with the azure B stain. Sections were then stained in

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azure B, washed with water and differentiated overnight with *t*-butyl alcohol (Flax *et al.*, 1952). Staining density with azure B was measured cytophotometrically at 545 nm. The mean values obtained for the percent transmission of a standard area of cytoplasm in liver were approximately 39.7 for the control birds and 26.8 for the treated birds. The decrease in percentage transmission was believed to have resulted from the presence of larger amounts of cytoplasmic RNA in the kestrels treated with Aroclor. A dose-dependent *in vitro* microsomal breakdown of estradiol to a more polar metabolite was observed in livers from kestrels dosed with either Aroclor tested. The effects of the two compounds were similar and were no different in male than they were in female birds. The conversions noted in Aroclor-dosed birds did not occur in untreated birds. Increased hepatic enzyme activity was found to be related to the increase in cytoplasmic RNA. The experimental findings suggest that the physiological actions of the tested PCBs were much like those of DDT and its metabolites and like the organochlorines, these industrial contaminants should be considered as a burden to the ecosystem.

71-0805. Fahim, M. S.; Bennett, R.; Hall, D. G. (Dept. of Obstetrics and Gynecology, U. of Missouri School of Medicine, Columbia, Mo. 65201). Effect of DDT on the nursing neonate. *Nature 228(5277): 1222-3; 1970.* (10 references)

Forty parturient Holtzman rats were injected i.p. with 0, 1, 5 or 25 mg of technical grade DDT/kg body weight 24 hr after delivery and daily for 21 days to determine the effect of DDT on the nursing neonate. After evaluation of growth and mortality rates, the animals were killed and calcium, potassium, magnesium, manganese, copper, iron and zinc in neonatal livers were determined by atomic absorption spectrophotometry. Growth rates of neonates of DDT-treated mothers were depressed proportionally to the amount of maternally administered drug. The mortality rates were 13, 32 and 100% of the neonates from mothers receiving doses of 1, 5 and 25 mg/kg/day, respectively. Hepatic assay revealed a significant increase in copper and a coincidental decrease in calcium. Recent studies have shown that DDT influences calcium metabolism in several avian species, stimulates hepatic microsomal activity in animals and humans, enhances metabolism of some drugs and increases urinary excretion of 6*beta*-hydroxycortisol. It is also indicated that the induction of avian hepatic enzyme activity by chlorinated hydrocarbons increases the metabolism of sex hormones both *in vitro* and *in vivo*. The structure of vitamin D is sufficiently similar to that of the sex hormone investigated to suggest that vitamin D metabolism might be accelerated by chlorinated hydrocarbons, thus affecting the absorption of calcium from the gastrointestinal tract. When compared on the basis of concentration and calculated as a fraction of the daily intake, cows excrete less DDT in lactational fluids than human females. This suggests that ill-effects may result from the exposure of human neonates to DDT through the mother's milk. This problem may be particularly significant in developing countries where the economy is largely agricultural and most children are breast-fed for 1 or more years. Further research concerning the dose-related effects of DDT on mineral metabolism in animal and human neonates is in progress.

71-0806. Findlay, G. M.; deFreitas, A. S. W. (Div. of Biology, National Res. Council of Canada, Ottawa 7, Canada). DDT movement from adipocyte to muscle cell during lipid utilization. *Nature 229(5279): 63-5; 1971.* (16 references)

Tissue samples from pigeons that had ingested 0, 3.101, 32.07 or 335.0 mg 14-C-DDT/bird and then were starved, were analyzed to determine the mobilization rate and the fate of the DDT quantitatively. After dosing, the birds were allowed to feed *ad libitum* for 3 days. At this time, six birds from each group were killed to determine the total body burden of DDT and its metabolites. The remaining six birds from each group were then maintained at 6 C with water but no food for 5 to 7 more days. The resultant 18 to 20% weight-loss corresponded to the 50 to 75% utilization of the total carcass lipid. The 14-C content of the extracted DDT and its metabolites was measured by liquid scintillation spectrometry. Starvation and cold did not alter the percentage of lipid or net DDT content of erythrocytes, plasma, liver or brain. There was a decrease of lipid in the heart muscle, but no change in the net DDT content. Conversely, the lipid content of the breast muscle, representative of the total body muscle, did not change, but the net DDT concentration increased significantly, demonstrating that starvation-induced changes in DDT content occur in adipocyte lipid and muscle tissue. Lipid utilization in the omental fat pad was accompanied by a net decrease in the total amount of DDT. This represented an increase in the concentration of DDT/g lipid, indicating that fatty acids were moving out of adipocytes more rapidly than DDT. As a result of stressing by starvation and cold, 0.45, 4.10 and 31.44 mg DDT/bird was moved from the adipose tissues into the muscle tissues of birds treated with 3.101, 32.07 and 335.0 mg DDT/bird, respectively. In the same dosage order, 0.672, 4.98 and 11.1 mg DDT/bird was irreversibly cleared from the system. That DDT is only relocated into the muscle tissue and not into the brain, liver, heart or blood, may protect DDT-sensitive tissues such as those of the central nervous system. DDT in the muscle tissues is not expected to cause cellular damage as it is probably located in the intracellular lipid droplets as seen in red muscle cells (Grinyer and George, 1969).

71-0807. Cory, L.; Fjeld, P.; Serat, W. (Dept. of Biology, Saint Mary's College, Saint Mary's College, Calif. 94575). Environmental DDT and the genetics of natural populations. *Nature 229(5280): 128-30; 1970.* (33 references)

The possibility of DDT residues being directly responsible for alterations in the genetic content of populations is discussed based on correlations between chromosomal changes in the fly *Drosophila pseudoobscura* as described over the past 24 years in the western U.S. and the distribution patterns of DDT residues found throughout the area. The configurations of inversions and combinations of inversions occurring in the third chromosome of *D. pseudoobscura* were named according to their area of discovery. The most relevant to this study are Arrowhead (AR), Chiricahua (CH), Pike's Peak (PP), and the standard or reference configuration (ST), of which the others were considered inversions. These different chromosomal forms were shown in laboratory tests to have differing genetic effects and can thus be regarded as a series of multiple alleles responding differentially to different selective

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