should be an important factor in the toxicological evaluation of this insecticide.

ACCUMULATION OF DDT IN THE BODY FAT

We had available for this study a number of dogs which had been receiving daily doses of DDT for periods of time varying from 138 days to two years. Since it was desired to continue the chronic experiments, these animals were anesthetized by intravenous injection of 40 mg/kg of sodium pentothal and samples of fat were taken from the peritoneal cavity under aseptic conditions.

The samples of fat were extracted with ether and the DDT determined by the Schechter and Haller[2] method. The quantities found in relation to dosage level, length of administration and form of dosage are shown in Table 1.

TABLE 1

ACCUMULATION OF DDT IN THE BODY FAT OF DOGS

Dog no.	Sex	Weight kg	Daily dose mg/kg	Form of administration	Days duration	DDT in fat mg/gm
M–166	f	8.9	10	soln.	747	0.080*
81–196	m	10.0	10	soln.	747	0.024
1–20	f	6.5	50	soln.	443	1.65
81–195	m	10.4	50	soln.	747	4.94
1–35	f	6.9	80	solid	443	0.39
M–171	m	10.3	80	solid	443	0.67
After discontinuing dose for 81 days						
1–59	f	7.3	80	soln.	138	0.013
1–61	m	9.3	80	soln.	138	0.00

* For purposes of comparison, the intravenous lethal dose of DDT is of the order of 0.04 milligrams per gram body weight.

Examination of these data reveals several significant facts. Storage of DDT in the body fat increases with level of administration. The fat accumulation is also profoundly influenced by the physical state of the DDT given. The toxicity observed in dogs with these dosages is similarly affected. No dogs, for example, have died from the 80 mg/kg/day dose of the dry solid DDT out of four, whereas the two dogs, 1–59 and 1–61, are the only ones of 16 that survived the 80 mg/kg/day dose of DDT dissolved in corn oil. One of these, we believe, would have also died had the dosage not been discontinued.

The fact that DDT disappears from the fat upon discontinuation of administration is demonstrated by examination of the data obtained in the case of the last two dogs in the table. Supporting evidence for appreciable fat storage in these animals at the time the treatment was withdrawn is the observed continuation of excretion of DDT metabolites in the urine of number 1–59 for 24 days and of number 1–61 for 16 days.

The distribution of DDT between subcutaneous fat and intraperitoneal fat was found to be equal in dog No. 1–35. This observation would indicate that the material is distributed uniformly throughout the fat depots in the body.

APPEARANCE OF DDT IN THE MILK

A third dog, 1–36, belonging to the group receiving 80 mg/kg/day solid DDT, had just weaned a litter during the course of this experiment. A small sample of milk was obtained upon each of two successive days and analyzed. DDT was found in amounts of 0.06 and 0.04 milligram per gram of milk, respectively.

A control dog, D–3, also with a litter was given a single 50 mg/kg dose of the ortho-para isomer of DDT, 2-o-chlorophenyl, 2-p-chlorophenyl-1,1,1-trichloroethane. Twenty hours later a sample of milk was obtained and found to contain approximately 0.05 milligram of the o,p-isomer per gram.

CONCLUSIONS

DDT in quantities of significance in its toxicological evaluation is stored in the body fat of dogs given daily oral doses. The storage increases with dosage level. Feeding oil solutions of DDT gives greater accumulation in the fat than does feeding the undissolved material. The accumulated DDT gradually disappears from the fat after discontinuation of administration.

The milk of lactating dogs receiving DDT or its ortho-para isomer contains appreciable levels of the respective compounds.

GEOFFREY WOODARD
RUTH R. OFNER
CHARLES M. MONTGOMERY
DIVISION OF PHARMACOLOGY,
FOOD AND DRUG ADMINISTRATION,
FEDERAL SECURITY AGENCY,
WASHINGTON 25, D. C.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW TEST FOR BLOOD ESTROGEN

IT is well known that the vagina of the rat remains closed until sexual maturity but opens earlier in response to the administration of estrogens. The test here reported depends upon this phenomenon, but administration is made locally and in the 21-day rat is by this method positive to extremely small dosage.

The procedure is extremely simple. The sample, in

volume of 0.01 or 0.02 cc, is injected subcutaneously near the region of the future vaginal orifice and observations are made once daily. The first indication of a positive reaction consists of a crescent-shaped transverse dimpling of the skin at the developing vaginal orifice. Openings appear at various points in the crescent and fluid oozes at such slits from gentle pressure.

On the average it requires 4 or 5 days for the vaginae of two out of three animals to open; but easily recognizable changes appear usually within 24 hours. If large doses are used, for example, 0.005 mgm of estradiol diproprionate[1] in 0.02 cc of oil, the vagina may open within 24 hours and definite changes, easily recognizable at a glance, have been noted within 17 hours.

One of the chief objects in developing a delicate test for estrogen seemed to us to lie in its possible applicability to assays of estrogen in blood. To our amazement, if our calculations are even approximately correct, we found the test to be almost unbelievably sensitive to blood estrogens, for as little as 0.02 cc of untreated finger blood from women was always positive, while that from four different men was always negative.

For most of the experiments to date 21-day-old female rats have been used; but in one experiment six females of one litter of 16-day-old rats were used with such success that it seems possible that the 16-day-old rat may become the animal of choice for these experiments. The young were injected and returned to their mother. Within four days all the injected females had open vaginae: the two which had received 0.0005 mgm of estradiol in 0.02 cc of oil and the three that had received 0.02 cc of midcycle female blood. Within 30 hours the dimpling was observable. The one control showed no change.

How delicate this test really is, in terms of blood estrogen, is seen in the following calculation. Taking the recent figures of Markee and Berg[2] as a basis we find the midcycle titre of estrogen in the blood to be roughly 0.005 mgm per liter or 0.000005 mgm per cc. A positive reaction, involving anatomical changes, is thus attained with five one hundred millionth of a milligram of estrogen—which, however, still contains some billions of molecules.

The test, it is to be noted, costs nothing, for the test animals are not sacrificed, remaining perfectly normal members of a colony. Furthermore, the time required to make and read the test is almost negligible.

CARL G. HARTMAN
J. LITTRELL

THE UNIVERSITY OF ILLINOIS

[1] Generously furnished by Dr. E. Oppenheimer, research director, Ciba Pharmaceutical Products, Inc.

[2] J. E. Markee and B. Berg, *Stanford Med. Bull.*, 2: 55, 1944.

NORELAC—A SUBSTITUTE FOR SHELLAC IN THE PRESERVATION OF SMOKED PAPER RECORDS

NORELAC (*Nor*thern *Re*gional *Lac*quer) is a new thermoplastic polymer developed at the Northern Regional Research Laboratory.[1] The properties of Norelac[2] and its solubility characteristics encouraged us to test its use as a substitute for shellac in the preservation of smoked-paper records, such as the kymograph records produced in physiological and pharmacological laboratories. The present war-time shortage of shellac has emphasized the need for a good substitute. Even when shellac is plentiful the preparation of a suitable coating for smoked records is something of a nuisance, and can not be done in a short time. A rather voluminous alcohol-insoluble residue must be allowed to settle and the supernatant solution decanted.

A few experimental trials with Norelac in a suitable solvent demonstrated readily that it can be substituted for shellac with complete satisfaction. A 5 per cent. solution of Norelac in a mixture of isopropyl alcohol and Skelly Solvent "C" (or naphtha) makes a good protective coating for a smoked-paper record. The record dries in ten minutes with a dull finish. If less than 5 per cent. of Norelac is used, abrasion marks are easily produced. If 10 per cent. of Norelac in isopropyl alcohol and Skelly Solvent "C" is used, the record dries free from tack in ten minutes with a glossy finish. The record can be given an intermediate degree of luster by the use of 7.5 per cent. of Norelac in a mixture of 75 to 85 parts of isopropyl alcohol and 25 to 15 parts of Skelly Solvent "C." The coating is applied to the smoked paper in the manner usually employed with alcohol solutions of shellac. The records coated with Norelac lie flat without curling, and can be stored flat or rolled without danger of sticking, marring or cracking. The addition of 1 to 2 per cent. of paraffin[3] will reduce any tendency to stick under unfavorable conditions of storage.

The sample of Norelac used had an iodine number of 89.2, indicating that there would be no danger of spontaneous combustion of stored records. A solution of Norelac has also been found suitable for protection of paper labels on reagent bottles and laboratory equipment.

It is convenient to prepare a stock 30 per cent. solution of Norelac in 99 per cent. isopropyl alcohol with the aid of heat, and to dilute as required for use to 5 or 10 per cent. or some intermediate concentration with a mixture of isopropyl alcohol and Skelly

[1] J. C. Cowan, A. J. Lewis and L. B. Falkenburg, *Oil and Soap*, 21: 101–107, 1944.

[2] Obtainable from General Mills, Inc., Minneapolis, Minn.

[3] J. C. Cowan, L. B. Falkenburg and A. W. Schwab, *Modern Packaging*, 17: 113–119, 1944.