Pesticide Residues in Fluid Market Milk

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DURING the fall of 1955, each of the 16 Food and Drug Districts submitted at least 100 samples of market milk to the Division of Antibiotics, Food and Drug Administration, Washington, D. C., where they were checked for residual antibiotics (*1*). In addition, 800 of these samples, 50 from each district, were analyzed by the Division of Food for pesticide residues.

Surveys in 1948, 1949, and 1951 had shown that traces of DDT could be found in about 25 percent of market milk samples. A fairly specific but somewhat long procedure, the Schechter-Haller method (*2–4*), was then available for detecting DDT, but more recently many other pesticides have come into use, and residues of lindane, technical BHC, methoxychlor, Rhothane, heptachlor, toxaphene, chlordane, members of the aldrin group, Perthane, Dilan, Lethane, and others might be encountered in milk. For most of these no specific test method exists. In addition to the group of chlorinated organics, trace residues of the organic phosphate pesticides, such as parathion, and Systox, might be found. A further complication was recognized from the start: Little is known about the metabolism of most of these products; some or all might degrade to unknown products of unknown toxicity.

Because of the dearth of specific methods, and because the application singly of the available ones to hundreds of samples would involve more work than a limited staff could handle, a bioassay "sort-out" test was applied. E. P. Laug of the Division of Pharmacology has perfected a bioassay with flies and applied it to the determination of DDT, lindane, endrin, and other pesticides (*5–7*). When only one toxicant is known to be present, the results can be made remarkably quantitative. When the toxicant is unknown, or a mixture of toxicants is present, fly mortality gives a positive indication of their presence and, barring synergistic effects, at least some idea of the amount. Thus, the fly bioassay appeared to be well suited to routine sort-out work.

The Division of Pharmacology collaborated in this survey by running the fly bioassays on the prepared extracts of the milk samples. There were 801 samples in all. The Schechter-Haller method was later applied to a number of samples which tested strongly positive by bioassay, and paper chromatographic techniques were applied to these latter samples in order to identify the residues that caused mortality to flies (*8*).

Experimental Studies

All the chlorinated organic pesticides are fat-soluble and if present can be presumed to occur in the fat component of the milk. Sample preparation must thus involve the separation of trace quantities of the various pesticides from a relatively great quantity of butterfat. The fly bioassay cannot be applied directly to extracted butterfat; extraneous oily residues of more than about 20 milligrams will suffocate the flies. Further, there is a limit to the sensi-

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tivity of the bioassay; as the test is conducted the flies will not react to, for example, much less than 5 micrograms of DDT. This amount of DDT would represent 0.05 p.p.m. for a 100-gram sample, and previous experience had shown us that amounts of this order were to be expected in some milk. Accordingly, it was necessary to develop techniques capable of separating microgram quantities of the various pesticides from 4 to 5 grams of butterfat. Good paper chromatograms likewise require that oily residues in the milk extracts be negligible.

Fly Bioassay

One-day-old flies from a special strain were employed. They were DDT-susceptible flies originally supplied by the Entomology Research Division of the Agricultural Research Service, U. S. Department of Agriculture, and maintained in the FDA Division of Pharmacology for about 10 years. Details of the bioassay may be summarized briefly: 1.30 gm. of flies (90 to 100 individuals), anesthetized with CO_2, were placed in flasks containing the milk residues plus 5.0 mg. oleic acid matrix and kept there overnight. A standard series, for example, 5, 8, 12.5, and 20 μg. p,p′ DDT plus 5.0 mg. oleic acid, and at least one blank flask containing only oleic acid, were also run (*5–7*). The percentage of mortality in all flasks was determined next morning.

It should be emphasized that mortality can be caused by factors other than the presence of pesticides; excessive fatty or waxy residues, while nontoxic in themselves, will suffocate the flies. Fortunately, flies exhibit a characteristic reaction to chlorinated pesticides, a well-defined dancing or jittering. In our experience, this characteristic symptom has as much, if not more, diagnostic value as the number of killed flies. The test was considered negative when the symptom was not observed.

In a number of samples, with no dancing or jittering, mortality was in excess of 10 percent. To avoid false positives such samples were reported as "negative, but excessive kill." It is possible that residual pesticides contributed to the high mortality and that characteristic toxic symptoms were missed during the overnight exposure periods.

Had only DDT been present, the mortality of the flies in the DDT standard flasks would have provided a means of assay. In this work, a "positive" indicated the presence of one or more unknown toxicants and, of course, some idea of the amount present. We had a few 100 percent kills. All positive fats were reserved for further work.

Chromatography

Paper chromatograms were run on the 160 milk fats, about 10 from each district, which exhibited the highest toxicity to flies. The general technique was that of Mitchell (*8*). Considerable preliminary work was necessary to determine the conditions under which all of the pesticides likely to occur in milk would separate on the chromatograms. Various solvent systems in varying proportions were tried: methanol-water, dioxane-water, acetone-water, and methyl Cellosolve-water. This latter system was finally selected, as it would separate DDT, which showed 2 spots for the 2 isomers of the technical product; DDE, the ethylenic decomposition product of DDT; DDD, or Rhothane; methoxychlor; lindane, or gamma BHC; technical BHC, which gave 2 spots, one due to the gamma isomer; and members of the aldrin group. Chromatographic separation of other pesticides—chlordane, toxaphene, heptachlor, Dilan, Perthane, and Lethane—was also investigated.

Schechter-Haller Method

One hundred sixty-nine of the milk fats exhibiting the highest fly kill, selected on the basis of about 10 from each district, were run by the Schechter-Haller colorimetric method. This method is not entirely specific for DDT; DDD, or Rhothane, the dichloro analog of DDT, interferes, as will Dilan to some extent. As the method is applied, methoxychlor and Perthane do not interfere, but various degradation products of DDT cause interfering colors (orange or pink). Technical DDT also contains about 25 percent of the o,p′ isomer which yields an orange color instead of the distinctive purple of the predominant p,p′ isomer. Thus, the test is only roughly quantitative; it was used mainly to confirm the chromatographic results. Actually, the colors produced ranged from a nor-

mal purple to intermediate pinkish "off-shades." However, as they were due to DDT or related compounds, any one of these colors was called positive.

Organic Phosphate Pesticides

Each of the 801 samples was checked for organic phosphate pesticide residues by an in vitro cholinesterase inhibition test. Here again preliminary testing of methods by means of recovery runs with various organic phosphate pesticides was necessary. For the test, portions of the $CHCl_3$ extracts of the milk were brominated. This converts most thiono and dithio forms of phosphate pesticides to potent inhibitors. Extracts were then tested for inhibition by a ferric hydroxamate colorimetric method which measures residual acetylcholine (*9*, *10*).

Results

On a countrywide basis the fly bioassay applied here revealed that 62 percent of the 801 milk samples contained pesticide residues (table 1). Most of the residues were in trace amounts that were not identified specifically. Thirty-eight percent of the samples were called negative; 11 percent of the total number caused fly kills in excess of 10 percent but were classed as negative because characteristic toxic effects were not observed.

In the chromatographic results from 160 samples, DDT, DDE, BHC, lindane, DDD, and methoxychlor were identified in the milk chromatograms (table 2). It was not unusual to find as many as 4 of these in 1 milk sample. Members of the aldrin group were not encountered; nor were chlordane, toxaphene, and heptachlor. Technical chlordane and toxaphene are complex mixtures and both chromatograph as streaks instead of spots. It is possible that very small amounts of these or their metabolites may have escaped detection because the attenuated streak might not have registered on the chromatogram. DDA, a known metabolite of DDT, was likewise not found in the milk samples. The repeated occurrence of DDD may be somewhat surprising, but we are certain that the identification was positive. It may occur per se or as an impurity in technical DDT. It has not been mentioned as a metabolite of DDT.

Usually the chromatograms were clear cut

Table 1. Results of fly bioassay on milk samples, Food and Drug Administration survey, 1955

Food and Drug District	Number of samples	Positive [1]		Negative [2]		Negative, but excessive kill [3]	
		Number	Percent	Number	Percent	Number	Percent
Atlanta	50	24	48	26	52	8	16
Baltimore	51	29	57	22	43	5	10
Boston	48	27	56	21	44	10	21
Buffalo	50	24	48	26	52	5	10
Chicago	52	30	58	22	42	7	14
Cincinnati	50	28	56	22	44	6	12
Denver	50	29	58	21	42	4	8
Kansas City	50	29	58	21	42	3	6
Los Angeles	50	37	74	13	26	6	12
Minneapolis	50	27	54	23	46	5	10
New Orleans	50	43	86	7	14	5	10
New York	50	31	62	19	38	9	18
Philadelphia	50	28	56	22	44	13	26
San Francisco	50	41	82	9	18	0	0
Seattle	50	37	74	13	26	2	4
St. Louis	50	32	64	18	36	1	2
Total	801	496	[4] 62	305	[4] 38	89	[4] 11

[1] Test called positive, regardless of mortality, if flies showed toxic symptoms.
[2] No toxic symptoms observed.
[3] No toxic symptoms, but kill greater than 10 percent.
[4] Average.

and easy to read; occasionally extraneous residues either streaked the chromatogram or produced diffused, fluorescent spots which, however, were distinct from the typical dark spots of silver formed by the chlorinated pesticides or their derivatives. An unidentified silver spot of low R_f value occurred in a large proportion of the samples, and a similarly unidentified spot of high R_f was occasionally noted.

An effort was made to concentrate the materials responsible for these two unknown spots and to check them for toxicity. A number of samples which gave both the high and low spots were composited and spotted on paper; after development the proper sections of the paper were leached and the residues exposed to flies. There was no detectable toxicity.

The ethylenic derivative of p,p′ DDT, or DDE, was often noted in the chromatograms. Possibly it occurs in milk as a metabolite of DDT, but it could have resulted from the breakdown of DDT during storage and during the analytical process. In fact, possible decomposition of pesticides during transport and storage of the samples was one of our major worries; slight acidities caused by souring of the milk samples, or the development of free acids in the stored fats, could destroy pesticides of the aldrin group. Possibly this is the reason these were not encountered.

In the 160 high mortality samples selected out of the 801 total, pesticides were found in the following order of incidence: BHC, 60 percent; DDT, 54 percent; lindane, 26 percent; DDD, or Rhothane, 24 percent; methoxychlor, 3 percent; DDE, either a breakdown product or metabolite of DDT, 36 percent. Usually the DDE spot occurred in conjunction with DDT, but occasionally it occurred alone.

About 53 percent of the high mortality samples, 89 out of 169, gave positive Schechter-Haller colors ranging from faint pink to deep purple (table 3). Read as p,p′ DDT (600 mμ), results ranged from a high figure of 1.46 p.p.m. on the basis of the original milk down to traces (about 3 μg per 100-gm. sample or 0.03 p.p.m.). The high figure is not accurate for DDT because DDD was also detected on the chromatogram of this sample.

In practically every case the colorimetric and chromatographic results were in at least qualitative agreement; sometimes the chromatogram registered a faint DDT spot for samples which gave no perceptible Schechter-Haller color.

In the early stages of the work we encountered two samples of milk which seemed to

Table 2. Chromatographic results on milk samples exhibiting the highest toxicity to flies, Food and Drug Administration survey, 1955

Food and Drug District	Number of samples	Number of samples containing:					
		DDT	DDD	DDE	BHC	Lindane	Methoxychlor
Atlanta	8	7	3	0	7	1	0
Baltimore	11	6	4	3	4	3	2
Boston	10	5	1	3	6	0	2
Buffalo	6	3	3	2	5	1	0
Chicago	10	4	0	3	4	6	0
Cincinnati	11	1	2	1	7	2	0
Denver	10	8	1	5	5	5	0
Kansas City	13	4	2	5	4	5	1
Los Angeles	10	10	9	9	4	2	0
Minneapolis	10	2	0	4	8	2	0
New Orleans	11	7	1	5	7	2	0
New York	10	1	0	2	6	4	0
Philadelphia	10	2	1	1	2	8	0
San Francisco	11	11	10	11	10	0	0
Seattle	10	10	2	2	10	0	0
St. Louis	9	5	0	1	7	1	0
Total	160	86	39	57	96	42	5
Percent of total		53.8	24.4	35.6	60.0	26.3	3.1

Table 3. Schechter-Haller test[1] on samples exhibiting the highest toxicity to flies, Food and Drug Administration survey of milk, 1955

Food and Drug District	Number of samples	Number positive	Percent positive	Range as DDT (p.p.m.)
Atlanta	11	7	64	trace–1.46
Baltimore	11	7	64	traces
Boston	11	6	55	traces
Buffalo	10	3	30	traces
Chicago	10	6	60	trace–0.16
Cincinnati	10	3	30	traces
Denver	10	8	80	trace–0.10
Kansas City	13	10	77	trace–0.32
Los Angeles	11	10	91	trace–1.20
Minneapolis	11	6	55	trace–0.04
New Orleans	10	2	20	traces
New York	10	3	30	traces
Philadelphia	10	1	10	0.06
San Francisco	11	11	100	trace–0.29
Seattle	10	4	40	trace–0.08
St. Louis	10	2	20	traces
Total	169	89	53	trace–1.46

[1] Reveals DDT and its decomposition products or metabolites, or both. DDD and Dilan are partial interferences.

NOTE: Trace represents approximately 3 μg. per 100-gm. sample or 0.03 p.p.m.

yield some anticholinesterase activity; however, as the work progressed to its conclusion, no more positives were found. Because of this, and especially in the light of more recent research on milk, these two seemingly positive results are now questioned. It seems unlikely that organic phosphate pesticide residues occur as such in present-day market milk.

Summary

1. More than 60 percent of 801 market milk samples collected in a countrywide survey in the fall of 1955 contained residues of chlorinated organic pesticides as indicated by the fly bioassay procedure used. Most of the residues were in trace amounts that were not identified specifically. The samples showing the highest kill of flies, about 21 percent of the samples, were examined by paper chromatography and by the Schechter-Haller method for DDT and related compounds.

2. One or more of the following, BHC, DDT, lindane, DDD (Rhothane), methoxychlor, and DDE, have been identified by paper chromatography in some of the residues.

3. Market milk may contain up to 1.5 p.p.m. DDT or related compounds or both. Results by chemical methods, such as the Schechter-Haller colorimetric method must be accepted with caution unless interferences are known to be absent.

4. Two obvious sources of contamination of milk with chloro-organic pesticides are residues on forage and contamination as a result of insecticide sprays either on the cows themselves or in the barns and dairies. It is not known which of these is mainly responsible for the contamination. Work designed to settle this point is now in progress in the Food and Drug Administration.

5. Organic phosphate pesticide residues were not detected in these milk samples by an in vitro cholinesterase inhibition test.

(A limited followup survey conducted during the winter of 1956–57 by Atlanta, New Orleans, Los Angeles, and San Francisco districts revealed little or no contamination of market milk with either BHC or DDT.)

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Details of the methods used in preparing the samples and subjecting them to bioassay and analysis will be supplied by the Food and Drug Administration upon request.

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Seventh International Cancer Congress

The Seventh International Cancer Congress, sponsored by the International Union Against Cancer, is scheduled for July 6–12, 1958, in London, England. Scientists and physicians are invited to submit previously unpublished or unreported papers on experimental or clinical aspects of cancer, or on cancer control. The deadline for registering without payment of late fee and for submitting papers is January 1, 1958.

A preliminary program and applications for registration and submission of papers may be obtained from either the Secretary General, Seventh International Cancer Congress, 45 Lincoln's Inn Fields, London, W.C. 2, England; or Dr. Harold F. Dorn, Secretary General, International Union Against Cancer, National Institutes of Health, Public Health Service, Bethesda 14, Md.

Travel allotments of about $530 each are available to a limited number of scientists and physicians residing in the United States to cover air tourist fares on a special 15-day overseas round trip; a 6-day per diem allowance; and reimbursement for registration fee. Investigators invited to take part in one of the symposiums before or after the congress may apply for additional funds.

Applications for travel allotments should be in the form of letters in sextuplet giving age, training, titles of publications in cancer or related fields, academic or professional title, and institutional affiliation. The letters should be countersigned by the department director or administrative officer. Applicants for travel allotments submitting papers to the congress must include 6 copies of an abstract not exceeding 250 words of each paper; those not planning to present papers should include 6 copies of a major, current investigative work. The letters and abstracts must be submitted before January 1, 1958, to the Chairman, USA National Committee on the International Union Against Cancer, 2101 Constitution Avenue, NW., Washington 25, D. C.

Applications for assistance toward travel expenses are entirely separate from applications for registration for the congress and for the submission of papers to the program committee. All applicants will be responsible for their own passports, visas, registration, travel arrangements, and hotel reservations.