The Fate of Aldrin and Dieldrin in the Animal Body

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With the widespread use of aldrin and dieldrin, information concerning their fate in the animal body is of considerable interest. Recent studies indicate that the conversion of aldrin to dieldrin takes place readily in the animal body and is fairly complete. It is assumed to occur in all animals inasmuch as it has been demonstrated in beef and dairy cattle, pigs, sheep, rats, and poultry. The change is apparently independent of the site of entry of the toxicant, as it occurs following either oral ingestion or subcutaneous injection. Dieldrin apparently is chemically unchanged in the body and is stored as such. The data include comparative analyses by colorimetric, bioassay, total organic chloride, and infrared spectroscopic methods.

THE WIDESPREAD USE OF ALDRIN (1,2,3,4,10,10 - hexachloro 1,4,4a,-5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene) and dieldrin (1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene) with their accompanying high mammalian toxicities has focused attention on the need for more information on the fate of these toxicants following their ingestion, absorption, or injection into the animal body. This paper describes the work that has been done thus far toward the identification of the metabolic products of aldrin and dieldrin in the animal body.

The study of the fate of insecticides in the animal body and the identification of their metabolic products has been the subject of considerable research in recent years. Of the synthetic insecticides, DDT has been studied the most extensively. The accumulation of DDT in dog tissues was studied by Finnegan, Haag, and Larson (*10*). They reported that following oral administration the toxicant is stored in all tissues, with the greatest accumulation in the fat. Other workers (*14, 15, 17, 28*) have shown that large quantities of DDT were stored in the fat of rats. A high accumulation of DDT in the mesenteric and kidney fat of sheep fed with DDT-treated alfalfa was reported by Harris and coworkers (*13*). DDT was readily found in the milk of dairy cattle soon after they had been subjected to DDT-treated feed (*1, 3*). Similar results were obtained following external treatment of the body of dairy cattle with DDT (*2, 4*). Mattson and coworkers (*18*) have shown the presence of both DDT and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) in human fat tissues. DDE was always found present in samples showing DDT deposits, and from this they suggest that DDT can be degraded to DDE in the human body.

Condron and coworkers (*5*) have reported on treating dairy barns and cows with lindane. Their findings indicated that the amount of lindane in the milk was relatively low and was reduced to less than 0.1 p.p.m. 1 week after treatment. van Asperen and Oppenoorth (*26*) reported that after subcutaneous, intraperitoneal or intravenous injection of lindane into albino mice a rapid breakdown of the toxicant was observed. They were unable to identify the decomposition products, however, and emphasized that chemical breakdown of the toxicants and detoxication need not parallel each other. Lehman (*16*) estimated that lindane was stored in the body at a level approximating the concentration of the dietary intake.

Perhaps the work that is more closely related to the findings discussed in this paper is that reported on heptachlor by Davidow and Radomski (*7, 22*) and Davidow, Radomski, and Ely (*8*). They have shown, rather conclusively, that heptachlor is oxidized to heptachlor epoxide and stored as such in the animal body. This same mechanism was demonstrated in tests in which cows, dogs, and rats had been maintained on heptachlor-containing diets. The epoxide was found in the milk almost immediately after administering heptachlor in corn oil to a test cow. The dissipation of the epoxide from the body following cessation of the heptachlor-containing diet proceeds at a rate faster than DDT but slower than lindane. Of particular interest is the fact that the epoxide is more toxic when administered intravenously to mice than is heptachlor.

Little is known of the fate of toxaphene or chlordan in the animal body because of their heterogeneous composition and the lack of suitable analytical methods for their analysis. Diephuis and Dunn (*9*) reported that toxaphene is stored unchanged in the fatty tissues of treated animals. Infrared examination of the tissue extract showed the presence of all of the absorption bands exhibited by toxaphene. Comparative bioassay and total chlorine analysis of the same tissue extract showed that the total organic chloride present in the fatty tissue has about the same toxicity to flies as an equivalent amount of toxaphene.

Analytical Methods

The main deterrent to any study of metabolic products is the lack of adequate analytical methods for their determination. The data and findings herein reported were developed over a 2-year period as the availability of suitable samples and the development of sensitive, accurate, and specific analytical methods permitted. It was not until the development of the following methods that a complete analytical picture could be obtained on the degradation and/or storage of aldrin and dieldrin in the animal body.

The determination of both aldrin and dieldrin can be accomplished with varying degrees of specificity and sensitivity with four types of analytical methods: specific colorimetric methods, bioassay, total organic chlorine, and infrared.

The aldrin colorimetric method was devised by Danish and Lidov (*6*) and later modified and improved by O'Don-

nell and coworkers (*20*). The method is specific for aldrin and has a sensitivity and reproducibility of 2 to 4 γ in animal tissues.

A colorimetric method, specific for dieldrin, was also used in these studies. This method, described by O'Donnell, Johnson, and Weiss (*19*), is sensitive and reproducible to within 5 to 8 γ of dieldrin in animal tissues.

Other methods, useful in the determination of both aldrin and dieldrin, were used in these tests. They were infrared, bioassay, and total chlorine. Each of these methods has added useful information in the identification of the metabolite of aldrin and the storage of dieldrin. The infrared technique (*11*), in spite of its poor sensitivity, was useful in the final identification of toxic components when sufficient amounts had been isolated. Bioassay, although nonspecific, yielded a measurement of total toxicity in a sample. Two bioassay techniques were used in this study: exposure of houseflies or pomace flies to a dried film (*23*) containing microgram quantities of toxicants extracted from animal tissues or products, and direct feeding of houseflies on milk (*24*) without prior extraction of the toxicant. Factors affecting the sensitivity and precision of the bioassay technique have been described in detail in the publications cited. While the total chlorine method (*3*) was not used in this laboratory for this study, significant data are presented which were obtained by the U. S. Department of Agriculture, Beltsville, Md., using this method.

All of the methods were, of course, tested for their applicability to the analysis of animal tissues and animal products prior to their use in these studies.

Indications of Change in Aldrin Molecule

Prior to the development of the phenyl azide colorimetric method for the determination of aldrin residues, the microbioassay technique was used exclusively for the analysis of toxic deposit levels in the animal body. In the analysis of tissues from aldrin-treated animals all toxic components thus determined were calculated and reported as aldrin. The data obtained indicated that aldrin accumulated in the body at a level comparable to that found in similar tests with other chlorinated hydrocarbon insecticides. With the development of the phenyl azide method, however, analyses specific for aldrin were possible. In the studies on aldrin that followed, both the bioassay and colorimetric methods were used for analysis. Occasionally, discrepancies were found when results obtained by the two methods were compared. These differences were too large to be attributed to experimental variations in either the bioassay or the chemical method.

Table I. Determination of Aldrin in Milk and Rat Fat

Material Analyzed	Amount of Aldrin in Diet	Period Animal on Treated Diet, Weeks	Aldrin Found, P.P.M. Bio-assay[a]	Colori-metric
Milk	None	..	..	0.02
	66.0 mg./day	2	1.7	<0.1
	63.5 mg./day	2	1.2	<0.1
	57.0 mg./day	2	1.1	<0.1
	30.2 mg./day	2	0.88	<0.1
Rat fat	None	..	..	0.06
	25 p.p.m.	24 and 28	46.8	2.5
	25 p.p.m.	24 and 28	53.2	2.8

[a] Bioassay of milk made by direct-feeding technique. The rat fat was analyzed by the dry-film bioassay technique following saponification and extraction of the toxicant

Indications of a change of the aldrin molecule in the animal body were shown in the analysis of milk samples taken from cows which had been fed known amounts of aldrin in corn oil orally for a 2-week period. Analysis of these samples was undertaken by both the aldrin colorimetric method and the direct-feeding bioassay technique. The data accumulated in this series of comparative analyses are summarized in Table I.

These data indicate a change of aldrin in the animal body. The phenyl azide procedure showed aldrin to be absent from the milk samples, while the bioassay indicated the presence of 1 to 2 p.p.m. of aldrin.

Further evidence of a change in the aldrin molecule in the body was obtained in a pair of similar, but unrelated, animal feeding tests conducted in 1952. In these tests, aldrin was incorporated in the diet of rats at similar dosages for approximately the same time interval. Inasmuch as the tests were conducted about a year apart, one set of tissues was submitted prior to the development of the aldrin colorimetric method and the other set subsequent to it. Results of analyses of the fat tissues from the animals in these tests have also been included in Table I.

The bioassay data show a high aldrin level in the subject tissues, while the results obtained colorimetrically indicate aldrin to be present at comparatively low levels. Even though both methods of analysis were not applied to the same tissues from each animal test, considerable significance can be placed in the data due to the similarity of the dietary intake and the length of the toxicant-ingestion period. These data, then, indicate the presence of a toxicant in the body of these test animals at a level 10 to 20 times higher than that shown to be aldrin by the specific colorimetric method.

On the basis of these findings it was apparent that aldrin was undergoing a structural change in a variety of situations which left the compound unreactive to phenyl azide but still highly toxic to the test insects. In view of the change of heptachlor to heptachlor epoxide in the animal, it seemed reasonable to assume that a similar epoxidation mechanism could change aldrin to dieldrin in the body. To determine if aldrin was, in fact, converted to dieldrin in the animal body and stored as such, the experiments discussed below were performed.

Identification of Metabolic Products of Aldrin and Dieldrin in Animal Body

During 1953 and 1954 a number of state and federal governmental institutions were engaged in the toxicological evaluation of aldrin and dieldrin. In connection with these studies, tissues from the animals under test were submitted to the Shell Development Co. for routine residue analyses. The availability of these samples offered an opportunity to carry out the toxicant-degradation studies described below.

Investigations of Fate of Aldrin

In 1953 the U. S. Department of Agriculture, Beltsville, Md., conducted a series of tests in which dairy cattle were subjected to a daily oral intake of 400 mg. of aldrin for an extended period. On the 41st day of the test, butterfat samples were prepared from the cream of the test animals and submitted for analysis.

The extract obtained following saponification of the butterfat sample was divided into two portions, one of which was analyzed by the phenyl azide colorimetric method, and the other by bioassay. The results obtained in these analyses (Table II) indicated that the aldrin level in the butter as determined by the phenyl azide method was less than 0.1 p.p.m. while that found by bioassay was 41 p.p.m.

In an effort to define more accurately the change that had taken place in the aldrin molecule, a portion of the sample prepared for bioassay was scanned in the infrared. Infrared analysis confirmed the absence of aldrin in the butter samples, but was ineffective in identification of the toxicant present. The lack of identification of aldrin in the sample

by infrared analysis can be considered significant, however, inasmuch as, based on the bioassay results, sufficient quantities of toxicant were present in the sample to ensure its detection if present. Assuming dieldrin to be present in the aldrin sample, its analysis by the specific dieldrin colorimetric method was undertaken. The results of this analysis showed a residue of apparent dieldrin in the aldrin sample of 49.5 p.p.m. (see Table II), which is not appreciably different from the amount of toxicant (41 p.p.m. expressed as aldrin) obtained by the bioassay method.

Analysis of a duplicate sample by the total organic chlorine method showed the presence of 38.6 p.p.m. of toxicant calculated as aldrin. Here again, good agreement with the bioassay result was obtained.

The data in Table II, therefore, indicated that dieldrin was present in the butter from aldrin-fed cows, although infrared analysis failed to provide positive identification.

An opportunity for further study of this problem presented itself with the receipt of sheep and beef tissues from a series of animal studies in which aldrin was injected subcutaneously into the neck of beef cattle and sheep at the rate of 50 mg. per kg. of body weight. One such injection was made in each animal. At the end of 30 days the animals were sacrificed and the tissues subjected to pathological and chemical examination. Fat tissues from these animals were supplied for aldrin degradation studies. The tissues were saponified and the resultant extract chromatographed through a column containing 5 grams of a 2 to 1 mixture of magnesium oxide (No. 2642, Westvaco Food and Machinery Co.) and Celite (Johns-Manville Co.), and 1 gram of Attaclay (Attapulgus Clay Co.). Results of analysis of the extract both before and after chromatography by the various methods were not significantly different. Analyses for the degradation product were made on aliquots of the same cleaned-up extracts by the bioassay, aldrin colorimetric, dieldrin colorimetric, and infrared methods. The results obtained in these analyses are summarized in Table II.

These data indicate that a large portion of the aldrin present in the body of these animals has been changed to dieldrin or to a toxic compound which, following reduction, reacts with phenyl azide. The fact that aldrin residues were found in the tissues adds significance to the data in that they eliminate any possibility of dieldrin being given by mistake to the aldrin-treated animals. A comparison of the data obtained by the three methods assumes considerable importance in that the sum of the residues found by the aldrin and dieldrin colorimetric methods approximates those found by bioassay. This indicates that the total toxicity in the extract is principally due to aldrin and dieldrin and not due to significant quantities of some other unidentified toxic component.

When the total toxicity in a sample, as determined by bioassay, is calculated, only a small difference is involved in expressing the results as parts per million of either aldrin or dieldrin. In a residue analysis by the dry-film bioassay method (*23*) the difference in toxicity of aldrin to dieldrin (approximately a 1 to 1 ratio) is relatively small.

Of greatest significance, however, is the fact that dieldrin was definitely identified in the extracts of all of the samples by the infrared method. Approximately 20 peaks, characteristic of dieldrin, were identified and their intensity was sufficient to mask all other peaks except those arising from the fat in the sample.

The results obtained through total chlorine analysis are also summarized in Table II. They agree in general with the results obtained by the other three methods.

Subsequent to the analysis of the beef and sheep fat described above, a number of samples of pig fat were obtained from pigs feeding on pastures treated with aldrin granules at rates approximating 2, 7, and 15 pounds of actual toxicant per acre. Each pig was allowed to graze unhindered on a treated pasture for a period of 77 days.

Analysis of the samples by the aldrin colorimetric, dieldrin colorimetric, bioassay, and total chlorine methods yielded the results also summarized in Table II.

These data confirm those described above and indicate that the change from aldrin to dieldrin has also taken place in the bodies of these animals. The fact that the toxicant levels in these tissues are markedly lower than those described above is probably due to the dissipation of deposits on the forage crop before the test was terminated. Thus, a lower dietary toxicant intake level was realized.

The fact that the change from aldrin to dieldrin in the animal body occurs rapidly has been shown to some extent by the data summarized in Table I on 12-hour milk samples. Further evidence that this is the case has been shown by the data obtained in the analysis of eggs. In these tests, hens were maintained on a diet of mash fortified with aldrin at the rate of 4 grams of toxicant per 100 pounds of feed (88 p.p.m.). This dietary intake was extended over a 4-month period. Eggs were collected daily during the test with a sample taken for analysis on the 104th day. Following saponification and chromatography the egg samples were subjected to comparative analysis as in the previous experiments.

The results (Table II) show that aldrin has been converted to dieldrin at high toxicant levels. Moreover, the presence of dieldrin was confirmed through the identification of 13 dieldrin peaks in the infrared spectra.

In summary, the data obtained by five analytical methods indicate that aldrin is changed to dieldrin in the animal body. These data assume added significance in view of the fact that the animal tissues and animal products from which these data were developed were submitted from independent animal studies. These tests also indicate that the change of aldrin to dieldrin probably takes place in all animals, inasmuch as similar findings were obtained on beef and dairy cattle, pigs, sheep, rats, and poultry.

The site of entry of aldrin into the animal body appears to have little effect on its change to dieldrin. The conversion was found to take place in pigs, rats, poultry, and dairy cows from digestive absorption following oral ingestion. A similar change was also found in beef cattle and sheep following subcutaneous injection.

The change from aldrin to dieldrin in the body apparently takes place readily. Dieldrin has been found in the milk of dairy cattle within 24 hours after ingestion of aldrin.

Stability of Dieldrin in Animal Body

It has been shown above that aldrin undergoes a rapid epoxidation to dieldrin in the animal body. This conversion becomes important only if dieldrin is the final product or is the stored product. In view of these findings it became desirable to determine the stability of dieldrin in the animal body. The following analyses were performed to develop this information.

In conjunction with the butter samples taken from aldrin-treated cows, which were discussed previously, similar samples taken from cows maintained on a dieldrin-containing diet were also received for analysis. The latter samples were prepared from the cream of cows which had been subjected to a daily dietary intake of 200 and 400 mg. of dieldrin, respectively, for a period of 110 days. Following saponification and chromatographic procedures, analysis was made on each sample by the dieldrin colorimetric, bioassay, and infrared methods. The results of these analyses are summarized in Table III.

These data again showed good agreement between results of the various analytical methods. The infrared analysis, in addition to giving an approximate toxicant level in the sample, yielded a scan giving five identifiable dieldrin peaks [8.5, 9.9, 11.0, 11.8 (epoxide peak), and 14.7 microns]. The data obtained, while not quantitative because of the lower sensitivity of the infrared method on these samples, were sufficiently accurate to substantiate the colorimetric

Table II. Comparative Analytical Results on Animal Products Obtained from Aldrin-Treated Animals

Type of Material	Intake of Aldrin	Period on Diet, Days	Site of Entry into Body	Toxicant Found by Various Analytical Methods, P.P.M.				
				Aldrin colorimetric	Dieldrin colorimetric	Bioassay[a]	Total chlorine	Infrared
Butterfat	None	...	...	0.06[b]	0.07[c]	..	..	...
	400 mg./day	41	Oral ingestion	<0.1	49.5	41	38.6	Absence of aldrin confirmed; no identifiable products
Beef fat	None	...	...	0.03[b]	0.04[c]	..	..	...
Sheep fat	None	...	...	0.05[b]	0.08[c]	..	..	...
Beef fat	50 mg./kg.	One injection; biopsy samples taken 30 days later	Subcutaneous injection	5.0	93.5	100	75	20 dieldrin peaks definitely identified; accounts for all recognizable peaks
			Subcutaneous injection	10.0	90.0	110	93	
Sheep fat	50 mg./kg.		Subcutaneous injection	7.5	42.5	55	..	
Pig fat	None	...	...	0.02[b]	0.04[c]	..	..	...
	Fed hay treated with aldrin at 2, 7, and 14 lb. per acre	77	Oral ingestion	<0.1	0.20	0.40	1.2	...
		77	Oral ingestion	<0.1	0.62	0.90	1.0	...
		77	Oral ingestion	<0.1	1.82	2.7	2.0	...
Eggs	None	...	...	0.02[b]	0.05[c]	..	..	...
	88 p.p.m.	104	Oral ingestion	<0.1	46.0	48.7	..	40.3 13 dieldrin peaks definitely identified

[a] Analytical results calculated as aldrin.
[b] Apparent aldrin content of untreated check sample.
[c] Apparent dieldrin content of untreated check sample.

Table III. Results of Comparative Analysis of Butterfat and Beef Fat for Dieldrin Residues by Four Analytical Methods

Type of Material	Intake of Aldrin	Period on Diet, Days	Site of Entry into Body	Toxicant Found, P.P.M.			
				Dieldrin, colorimetric	Bioassay[a]	Total chlorine	Infrared
Butterfat	None	...	...	0.06[b]	..	..	..
	400 mg./day	110	Oral ingestion	95.0	95	72.6	96–102
	200 mg./day	110	Oral ingestion	49.6	30	46.4	Five dieldrin peaks definitely identified
Beef fat	None	...	...	0.07[b]	..	..	...
	25 mg./kg.	One injection; biopsy samples taken 30 days later	Subcutaneous injection	85	86	89	70 20 dieldrin peaks definitely identified

[a] Results calculated as dieldrin.
[b] Apparent dieldrin content of untreated check sample.

and bioassay results. Analysis by the total chlorine method yielded results in good agreement with those obtained by other methods and have been included in Table III.

Inasmuch as the dieldrin found by the colorimetric and infrared methods accounts for all of the toxicity in the sample, if any slight change had taken place in the dieldrin molecule the resulting product was probably nontoxic.

Similar findings were obtained on beef fat. In these tests as in those on aldrin described previously, a dieldrin solution was injected subcutaneously into the neck of beef cattle at the rate of 25 mg. of toxicant per kg. of body weight. One such injection was made to each animal. The animal from which this sample was taken was slaughtered 30 days after injection and the fat tissues were submitted for analysis.

Following preliminary cleanup by direct saponification and chromatography, as described above, the sample was analyzed by the dieldrin colorimetric, bioassay, total chlorine, and infrared methods. The data obtained (Table III) show good agreement between methods with the identification of approximately 20 dieldrin peaks in the infrared scan. Here again it appears that dieldrin accounts for all the toxicity present in the sample.

On the basis of the results obtained in the tests shown above, it appears that dieldrin is stored unchanged in the animal body. Further, any change undetected up to this time is assumed to be concerned with a relatively small amount.

Discussion

Knowing that aldrin is changed to dieldrin in the animal body, the question immediately arises concerning the implication of these findings. The change of a toxicant into another component within the body need not necessarily be detrimental to the use of the material. If adequate analytical methods are available for the determination of the degradation products, the hazard from use of the material can be measured. Such is the case with aldrin. Accurate, sensitive, and specific analytical methods are available for the determination of both aldrin and its major metabolite, dieldrin. The fact that aldrin largely changes to dieldrin rather than to some unknown constituent is fortunate, since a large amount of toxicological data have been amassed for dieldrin (*12*, *21*, *25*, *27*). The potential hazard from an unknown material is thus greatly reduced.

Conclusions

On the basis of the data summarized in this report the following conclusions have been drawn.

Aldrin is largely and readily converted to dieldrin in the body of beef and dairy cattle, pigs, sheep, rats, and poultry.

The change of aldrin to dieldrin is apparently independent of the site of

entry of the toxicant into the body, as it was found following oral ingestion and subcutaneous injections.

Dieldrin is stored unchanged in the body and is recovered as such from animal products and body tissues.

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PESTICIDE RESIDUES

Malathion in Milk and Fat from Sprayed Cattle

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Entomological studies have indicated that malathion, S-(1,2-dicarbethoxyethyl)O,O-dimethyl dithiophosphate, is an effective insecticide against livestock pests, and toxicology studies have shown that it may be safely applied to livestock. However, before it could be recommended for use on beef and dairy cows it was necessary to determine whether its use as a spray would cause contamination of meat or milk. Hereford cattle were sprayed 16 times with 0.5% malathion. Fat samples taken 1 week after the last spraying contained no detectable amounts of the insecticide. When dairy cows were sprayed with 0.5 and 1.0% malathion the insecticide was present in all milk samples taken 5 hours after spraying, ranging from 0.08 to 0.36 p.p.m. Only traces were present 24 hours after spraying and samples taken 3 and 7 days after spraying were free of contamination.

Small-scale experiments with malathion against insects affecting livestock have been made during the past 2 years by entomologists of the Kerrville, Tex., laboratory. Their results have indicated that 0.5% concentration of malathion kills flies, ticks, and lice, but that its residual effect does not persist as long as that of the chlorinated hydrocarbon insecticides. Veterinarians at Kerrville found that even such susceptible animals as baby calves appeared unharmed by single sprayings of 0.5% malathion (*3*).

As malathion was indicated to be toxic to a number of livestock insects but not acutely poisonous to cattle, experiments were conducted in cooperation with the American Cyanamid Co. to investigate some toxicological aspects of its use. One phase of that study was to establish whether repeated sprayings would result in a deposition of the insecticide in the fat of cattle. Another phase was