INFLUENCE OF PROCESSING ON THE DEGRADATION OF PESTICIDES IN MEAT PRODUCTS

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SUMMARY

The influence of processing on the degradation of C-14 labelled DDT, γ-hexachlorocyclohexane (γ-HCH) and monolinuron (MLN) was investigated. In culture media micrococci and yeasts metabolised DDT, mainly to DDD, within two weeks, whereas γ-HCH and MLN were negligibly degraded. Only small amounts of the pesticides were metabolised by lactobacilli.

Meat of rabbits fed with C-14 labelled pesticides was used to prepare various meat products. In dry sausages with nitrite-curing salt (NCS) up to 60 % residual DDT and 70 % γ-HCH were recovered. In the presence of a starter culture and nitrate the rate of degradation was lower, 76 % DDT (after 38 days) and 77 % of γ-HCH (after 30 days) of the initial concentrations were found in the final products. In M. long. dorsi *61 % DDT and 80 % γ-HCH were still present after curing. In cured, cooked meats 87 % DDT but only 35 % γ-HCH of the initial amounts were left. In the case of MLN about 50 % of the radioactivity was removed by curing, hot-smoking or cooking; only 2 % of the radioactive material was extractable. The enzymic degradation of DDT, γ-HCH and MLN, respectively, by muscle mitochondria (released after freezing) amounted to about 10 % on average.*

In liver sausage mixtures with sodium chloride, DDT and γ-HCH were metabolised to a greater extent than in mixtures containing NCS or NCS and sodium ascorbate (NaASC).

A modified ascorbic acid oxidation system did not enhance the degradation of the pesticides investigated to a significant extent in a cervelat type sausage.

ABBREVIATIONS

2,2-bis(*p*-chlorophenyl)acetic acid	DDA
2,2-bis(*p*-chlorophenyl)ethanol	DDOH

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4,4′-dichlorobenzophenone	DCB
2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane	DDD
1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	DDT
1-chloro-2,2-bis(*p*-chlorophenyl)ethylene	DDMU
1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene	DDE
2,3,5-trichlorophenol	2,3,5-TCP
2,4,5-trichlorophenol	2,4,5-TCP
γ-1,2,3,4,5,6-hexachloro-cyclohexane, lindane	γ-HCH
1,2,3-trichlorobenzene	1,2,3-TCB
1,2,4-trichlorobenzene	1,2,4-TCB
1,2,3,4-tetrachlorobenzene	1,2,3,4-TetCB
1,2,4,5-tetrachlorobenzene	1,2,4,5-TetCB
pentachlorobenzene	PentCB
γ-2,3,4,5,6-pentachloro-cyclohex-1-ene	γ-PCCH
chlorobenzenes	CB
chlorophenoles	CP
3-(4-chlorophenyl)-1-methoxy-1-methylurea,Monolinuron,Aresin	MLN
p-chloro-aniline	pCA
nitrite-curing salt	NCS
sodium ascorbate	NaASC

INTRODUCTION

The importation of contaminated feeding material, mainly from developing countries, results in an accumulation of persistent pesticides in animals. Therefore, technological procedures in food production should be developed to reduce the content of pesticides hazardous to public health in meat products. The present investigations deal with the possibility of influencing the content of DDT, γ-HCH and monolinuron (MLN) in meat products by various technological procedures. The abbreviations for DDT and its metabolites refer to the *p,p′*-isomers. Abbreviations for other compounds are included in the above list.

Because only limited amounts of C-14 labelled MLN were available the experiments with this pesticide had to be restricted.

METHODS AND MATERIALS

Muscle and fatty tissue of rabbits contaminated with the pesticides in question were used. In the case of MLN, corn treated with the C-14 labelled compound was fed to the animals for five days. In addition, $1{\cdot}4 \times 10^7$ dpm C-14 ring labelled MLN in

olive oil was administered by a stomach tube two days before slaughter. The MLN-contaminated plants, as well as the C-14 labelled compounds, were kindly provided by Dr Suess, Isotope Laboratory of the Bavarian Institute of Soil Research and Agriculture, Munich. Muscle and fatty tissue were stored and processed as described previously (Mirna & Coretti, 1974*a*, 1974*b*; Mirna *et al.*, 1975). In some experiments, the pesticides were added directly to the ground meat or sausage mixtures.

Determination of the pesticides

The analytical procedures for the determination of DDT, γ-HCH and its metabolites have been described in previous publications (Mirna & Coretti, 1974*a*, 1974*b*; Mirna *et al.*, 1975). The R_f-values and recovery rates for γ-HCH and its metabolites are summarised in Table 1.

MLN was determined as follows. The sample (10 g) was homogenised in a 100 ml centrifuge bottle with 50 ml of 80 % acetone. After centrifugation the supernatant fluid was collected and the residue extracted again in the same way. The combined supernatant fluids were stored for at least one hour in a refrigerator so that the fat particles could solidify. The cloudy extract was poured through a wet filter, the clear filtrate diluted with 400 ml water and 150 ml saturated sodium chloride solution and

TABLE 1
R_f VALUES AND RECOVERY RATES FOR TLC OF γ-HCH AND ITS METABOLITES

Solvent	*Substance*	R_f *value*	*Recovery rate* (%)
(a)	2,3,5-TCP	0·00–0·02	37
	2,4,5-TCP		35
	PentCP		29
	γ-HCH	0·10	94
	γ-PCCH	0·54	—
	1,2,3-TCB	0·59	72
	1,2,3,4-TetCB	0·65	49
	1,2,4-TCB	0·67	65
	1,2,4,5-TetCB	0·72	75
	PentCB	0·73	8
(b)	2,4,5-TCP	0·49	
	PentCP	0·59	
	2,3,5-TCP	0·61	
	γ-HCH	0·83	
	1,2,4-TCB	0·86–0·88	
	1,2,3-TCB		
	1,2,3,4-TetCB		
	1,2,4,5-TetCB		
	PentCB		
	γ-PCCH	0·92	

Silica gel HF_{254} (Merck)-aluminium oxide (Woelm, neutral)-plates (42:18).
Thickness of the layer: 0·5 mm.
Solvent (a): *i*-Octane-Dioxane-Dimethylformamide (98:1:1, vol/vol).
Solvent (b): Benzene-Dioxane-Glacial Acid (90:10:2, vol/vol).

then extracted three times with ether (first with 100 ml and then twice with 50 ml aliquots). The combined ether extracts were washed twice with 10 ml water and dried over sodium sulphate.

After filtration, toluene (1 ml) was added and the extract concentrated to approximately 1 ml on a rotatory vacuum evaporator. The remaining solution was transferred to a small calibrated tube and diluted to 3 ml with toluene. Portions were used for the determination of the extractable activity and for thin-layer chromatography (TLC). The positions of the labelled compounds on the plates were

TABLE 2
R_f VALUES AND RECOVERY RATES FOR TLC OF MONOLINURON (MLN) AND *p*-CHLORO-ANILINE (pCA)

Substance	R_f *value*	*Recovery rate* (%)
MLN	0·13	82
pCA	0·23	69

Silica gel HF_{254}.
Thickness of the layer: 0·5 mm.
Solvent: toluene-chloroform (75:25, vol/vol).

localised by means of a TLC-Scanner II (Berthold-Friesecke). The radioactivity was measured with a Liquid Scintillation Spectrometer (SL 30, Intertechnique). Quenching was corrected by external standardisation. Table 2 shows the R_f-values and the recovery for MLN and the main metabolite, *p*-chloro-aniline (pCA).

Determination of the total radioactivity

The determination of the total radioactivity was carried out by means of a Tri-Carb Sample Oxidiser (Packard, Model 306). Carbon dioxide was trapped by Carbosorb and Perma Fluor V was used as scintillator for the liquid scintillation counting (LSC) measurement. Incomplete combustion was obtained with 0·5 g samples. Carbonaceous particles were deposited in the combustion flask and this resulted in a yellowish counting solution. These difficulties could be eliminated by means of the following modification. Approximately 0·5 g of the sample was smeared on the wall of a small cellulose cone and covered with about 0·1 g cellulose powder. After positioning the cone in the ignition basket, 0·2 ml 'Combust-Aid' (an organic solvent mixture used to better ignite the sample) was added. Between the last coil of the ignition basket a piece of a platinum-net (2 × 2 cm, mesh size 0·25 mm) was fixed. The platinum-net acts as a catalyst in a kind of afterburner to remove the volatile crack products.

This modified ignition device works very efficiently. By addition of 1×10^3 to 2×10^3 dpm C-14 DDT to 0·5 g of a sausage mixture the recovery amounted to $100 \pm 2\%$.

RESULTS

Degradation of DDT, γ-HCH and monolinuron by microorganisms

Micrococci, lactobacilli, and *Debaryomyces kloeckeri* were tested for their ability to degrade DDT, γ-HCH and MLN, respectively. A freezedried mixture of micrococci and lactobacilli is commercially available as Duploferment (R. Müller & Co., Giessen). In addition to these bacterial strains, yeasts are regularly present in dry sausage (Coretti, 1972; Rossmanith *et al.*, 1972).

Microorganisms of a starter culture (Duploferment) were grown in Trypticase Soy Broth (TSB, Difco). The vials containing the test medium and 2×10^4 to 5×10^4 dpm of the C-14 labelled pesticide were inoculated with a 20 h culture of the bacterial strain and incubated at 30 °C. In this medium, adjusted to pH 7·3, only micrococci developed satisfactorily.

At certain intervals a carrier solution (a mixture of the metabolites) was added to the vial and the sample centrifuged for 5 min at 2500 rpm. The supernatant fluid was decanted and the residue treated in the same way with 50 ml of a physiological saline solution. In the experiments with DDT and γ-HCH the combined supernatant fluids were extracted with petroleum ether (once with 100 ml and twice with 50 ml

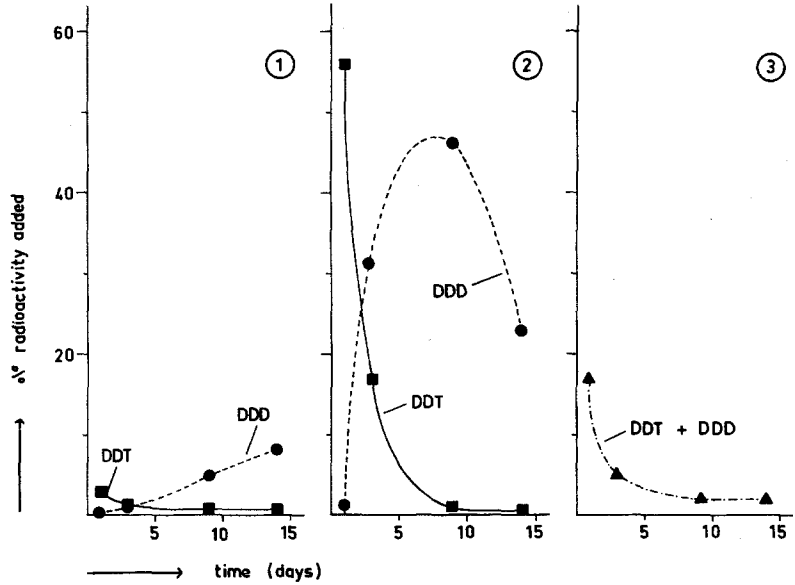

Fig. 1. Degradation of C-14 labelled DDT by microorganisms in starter culture. Medium: Trypticase Soy Broth (Difco). Incubation temperature: 30 °C; pH 7·3. (1) Radioactivity extractable from the centrifuged cells. (2) Radioactivity extracted by petroleum ether from the centrifuged liquid medium. (3) Radioactivity extracted by petroleum ether after acid hydrolysis (pH < 1; 10 h at 20 °C) of the aqueous phase sub item (2).

aliquots). The remaining aqueous phase was acidified to *ca.* pH 1, kept at 20 °C for at least 10 h for hydrolysis, and extracted again with ether. The extraction of MLN was carried out as described in the section headed 'Methods and Materials'. The residue from the centrifugation was dried in a stream of nitrogen, 1 ml of acetone was added and left standing overnight. The solution was then transferred to a 3 ml test tube with about 2–3 ml of dichloromethane, concentrated to approximately 0·2 ml on a rotatory vacuum evaporator, and applied to a plate for separation by thin-layer chromatography. The results of the experiments with Duploferment and yeasts (*Debaryomyces kloeckeri*) respectively are illustrated in Figs. 1 and 2.

Micrococci, as well as *Debaryomyces kloeckeri*, degraded DDT, mainly to DDD, during two weeks' incubation in liquid culture media. Small amounts of the pesticide and its metabolites were extractable after acid hydrolysis. During the experiments an increasing amount of radioactivity was deposited in the sediment of the centrifuged cells. In some cases as well as DDD, 1–2 % DDE and DDMU and less than 1 % of DDA, DDOH and DCB were present in the various fractions.

In the analogous experiment with γ-HCH about 30 % of the pesticide was metabolised after 14 days. Very small amounts of chlorobenzenes (< 1 %), chlorophenols (max. 2 %) and 2 % to 9 % of unidentified compounds were found as

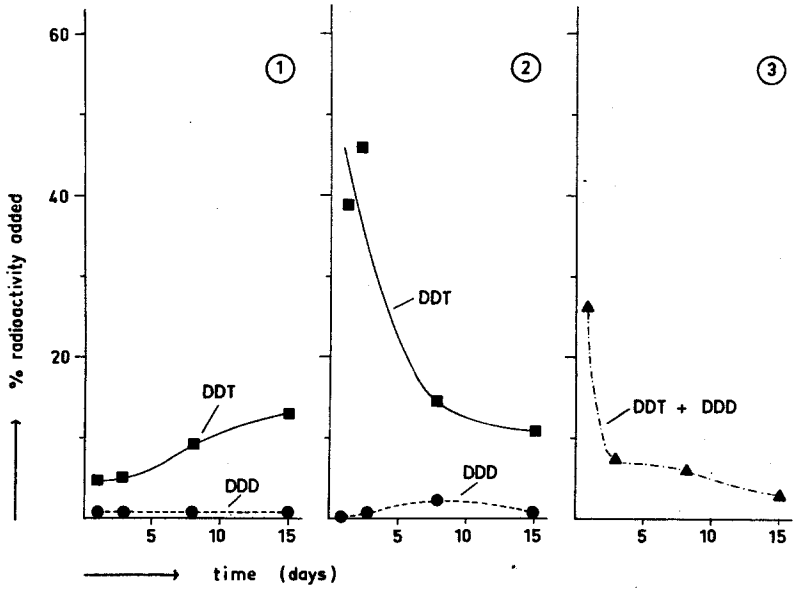

Fig. 2. Degradation of C-14 labelled DDT by *Debaryomyces kloeckeri*. Medium: Glucose-peptone-broth (SABOURAUD, Merck). Incubation temperature: 25 °C; pH 5·7. (1) Radioactivity extractable from the centrifuged cells. (2) Radioactivity extracted by petroleum ether from the centrifuged liquid medium. (3) Radioactivity extracted by petroleum ether after acid hydrolysis (pH < 1, 10 h at 20 °C) of the aqueous phase sub item (2).

degradation products. Radioactivity was not detected in either the extract obtained from acid hydrolysis or in the centrifuged cells.

The rate of degradation of MLN by Duploferment and yeasts amounted to only 10 to 20 %. Not more than 2 % pCA and 2 to 4 % of unidentified compounds were present. Less than 1 % of the radioactivity was deposited in the centrifuged cells. Hydrolysis with HCl was not carried out with regard to the formation of artefacts from MLN. Two strains of an atypical lactobacillus were grown in Man Rogosa Sharpe (MRS)-medium (Difco). Both the tested lactobacilli were rather ineffective in metabolising DDT. This was probably due to the inhibition of the degrading enzyme systems by a marked decrease in the pH value from 6·5 to 4·3 and 4·0, respectively. Under identical conditions the degradation of γ-HCH was approximately 10 % only.

Degradation of DDT, γ-HCH and monolinuron by tissue enzymes

Some of the enzyme systems present in the tissues are fixed in the mitochondria. Membranes of the cells are partly deteriorated by freezing, which results in a release of enzymes into the sarcoplasma after thawing (Hamm & Körmendy, 1969; Hamm & El-Badawi, 1972). Therefore, in processing frozen meat, a more intensive reaction of the released mitochondrial enzymes with pesticides might occur.

In ground beef after a two-day storage period at 1–2 °C, the residual DDT amounted to 94 %, γ-HCH to 89 % and MLN to 96 %. A preceding storage at −20 °C for eight days caused only small changes in the residues of DDT (91 %) and γ-HCH (84 %) but MLN was notably decreased to 82 %. In the experiment with DDT about 3 % DDE and less than 1 % DDOH, DCB, DDD and DDMU were found. Among the degradation products of γ-HCH about 4 % CB and two unidentified compounds in amounts to 9 % and <1 % were observed. The metabolisation of MLN resulted in the formation of <1 % pCA and two unidentifiable substances in amounts of 3 % and <1 %, respectively.

Degradation of DDT, γ-HCH and monolinuron in meat products

Meat from rabbits fed C-14 labelled pesticides was used. The degradation of DDT, γ-HCH and MLN in dry sausages, cured, hot-smoked and cooked meat, as well as by liver enzymes, was studied. Two sausage mixtures, A and B, were manufactured, filled into casings (Naturin R 2, diameter 55 mm) and aged at 20 °C and 95 % relative humidity. A three-day treatment with cold smoke followed. Mixture A was prepared with 2·8 % curing-salt (NCS) and mixture B with sodium chloride, 600 ppm KNO_3 and Duploferment. The pesticide residues in the final products consisted mainly of unchanged DDT and γ-HCH, respectively (see Table 3).

DDT as well as γ-HCH were degraded to a greater extent in sausage mixture A (with NCS) than in mixture B inoculated with the starter culture. Among the metabolites DDD (4–5 %) predominated, followed by DCB and DDE, and only

TABLE 3

DEGRADATION OF DDT AND γ-HCH IN DRY SAUSAGES AFTER STORAGE; VALUES CALCULATED ON A DRY-MATTER BASIS

Mixture A: 2·8% nitrite-curing salt (NCS). Mixture B: 2·8% sodium chloride, 0·06 % KNO_3 and Duploferment.

Pesticide	*Sausage mixture*	*Storage time (days)*	*C-14 activity (%) of the total activity*[a]							
			DDT	*DDA*	*DCB*	*DDD*	*DDE*	*γ-HCH*	*CB*	*CP*
DDT	A	38	60	<1	1	4	2	—	—	—
	B	38	76	<1	3	5	1	—	—	—
γ-HCH	A	30	—	—	—	—	—	71	<1	<1
	B	30	—	—	—	—	—	77	<1	<1

[a] Determined by combustion.

rather small amounts of DDA (<1 %) were found. Less than 1 % CB and CP, the metabolites of γ-HCH, were present; no γ-PCCH was detected.

M. long. dorsi of rabbits were cured at 5 °C for 10 days. The brine contained 10 % sodium chloride and 0·015 % KNO_3; the proportion of brine to meat was 3:1. The results of the investigations on DDT, γ-HCH and MLN are summarised in Table 4.

The samples were investigated immediately after curing, after hot-smoking (2 h at 60 °C) and after cooking (1·5 h at 100 °C). In the experiments with DDT and γ-HCH most of the remaining radioactivity consisted of the unchanged pesticide. The

TABLE 4

CHANGES IN THE CONTENT OF DDT, γ-HCH AND MLN IN FRESH AND PROCESSED MEAT (*M. long. dorsi* OF RABBITS). (VALUES CALCULATED ON A DRY-MATTER BASIS)

Pesticides/metabolites		*C-14 activity (%) in the meats*[a]			
		Fresh	*Cured*	*Cured, hot-smoked*	*Cured, cooked*
DDT	*pH:*	6·0	6·3	5·9	6·8
	moisture (%):	70·5	76·9	61·7	69·5
Total activity		100	66	81	94
DDT		92	61	75	87
DDD		4	3	4	4
DDE		2	1	2	2
DCB		2	<1	0	<1
DDMU		0	<1	0	<1
γ-HCH	*pH:*	5·7	5·8	5·1	6·3
	moisture (%):	64·4	73·3	63·1	65·1
Total activity		100	87	91	46
γ-HCH		98	80	88	35
CB		2	2	<1	2
CP		<1	<1	<1	<1
MLN	*pH:*	5·6	5·2	5·3	5·8
	moisture (%):	63·0	68·1	54·8	57·2
Total activity		100	44	52	56
Extractable activity		2	2	2	2

[a] Total activity determined by combustion.

treatment most effective in eliminating DDT was curing; the residual pesticide amounted to 61 %. After hot-smoking 75 % DDT, and after cooking 94 % DDT, were still present in the meat products. As metabolites, mainly 3 to 4 % DDD, 1 to 2 % DDE and 1 to 2 % DCB and DDMU were found; DDA and DDOH were not detected.

The corresponding values determined experimentally with γ-HCH were 80 %, 88 % and 35 %, respectively. Thus, by cooking, 65 % γ-HCH was removed. Owing to the small amounts of radioactive material extractable in the experiments with MLN, no further identification could be made. In the experiments with pesticides no measurable radioactivity could be detected in the extract from the brines. Of the total radioactivity in the meat, approximately 5 % DDT, 4 % γ-HCH and 11 % MLN were found in the extracts from the different meat broths.

Degradation of DDT and γ-HCH by enzyme systems of the liver

Various enzyme systems are localised in the liver. Therefore it was of interest to study the influence of liver enzymes on the degradation rate of pesticides. The influence of food additives such as sodium chloride, nitrite-curing salt and sodium ascorbate (NaASC), as well as that of the heating procedure was also investigated (see Table 5).

Storage at 2–3 °C for two days should establish whether the rate of degradation could be increased by a prolonged incubation with the enzymes present. The pesticides residues consisted mainly of unchanged DDT and γ-HCH, respectively. Only small amounts of DDD, DDE, DDMU and DCB were found; DDA and

TABLE 5

INFLUENCE OF LIVER ENZYME SYSTEMS (BOVINE LIVER) AND STERILISATION ON THE DEGRADATION OF DDT AND γ-HCH

No./additives	*C-14 activity (%) of the total activity*[a]									
	DCB	*DDD*	*DDT*	*DDMU*	*DDE*	*γ-HCH*	*CB*	*CP*	$R_f = 0{\cdot}41$[b]	$R_f = 0{\cdot}15$[b]
A 1·6 % NaCl	<1	<1	90	1	2	85	0	0	2	2
B	0	0	59	0	0	71	0	0	<1	0
C	0	<1	69	0	0	16	<1	<1	<1	0
A 1·6 % NCS	0	0	80	0	1	84	0	0	2	5
B	0	0	91	0	0	71	0	1	<1	24
C	0	0	78	0	0	61	<1	20	3	0
A 1·6 % NCS + 500 ppm NaASC	0	0	82	0	0	87	0	<1	2	0
B	0	<1	100	0	0	81	<1	14	2	0
C	<1	0	89	0	0	88	1	1	4	0

[a] Determined by combustion.
[b] R_f values for solvent (a) (see Table 1).
A: sausage mixture investigated immediately after processing.
B: sausage mixture under (A) investigated after a 2-day storage at 2–3 °C.
C: sausage mixture under (A) filled in cans, 30 min sterilised at 100 °C, investigated after a 20-day storage at 5 °C.

DDOH were not present. In most cases, less than 1% CB was detectable as a metabolite of γ-HCH, whereas striking variations were observed in the content of CP (up to 20%) and two unidentified substances with $R_f = 0{\cdot}41$ (up to 4%) and $R_f = 0{\cdot}15$ (up to 24%), respectively. The amount of pesticide residues extractable from manufactured sausage mixtures containing added sodium chloride was generally lower than that from products containing either NCS or NCS and NaASC. This was especially true for the sterilised products. Presumably, groups which might react with the pesticides, chiefly by the formation of conjugates, are inhibited by reaction with nitrite.

Model experiments with ascorbic acid oxidation system

In a modified ascorbic acid oxidation system, where EDTA was substituted by trisodium citrate, 83% DDT, 63% γ-HCH and 52% MLN remained unchanged after a 12-h treatment at 20 °C. In protein-containing substrates, where two portions of the phosphate buffer pH 6·7 were replaced by blood, 47% DDT, 65% γ-HCH and 84% MLN were still present.

By adding NaASC and ferro-sulphate to a sausage mixture of the cervelat type, the degradation of the pesticides investigated was enhanced very little as compared with the control mixture after a 22-day storage period.

DISCUSSION

Microbial degradation of DDT, γ-HCH and MLN was studied in culture media with micrococci, lactobacilli and *Debaryomyces kloeckeri*. Micrococci, as well as the yeast, were able to metabolise DDT, mainly to DDD, within two weeks. Small amounts of this pesticide and its metabolites were extractable after acid hydrolysis. Furthermore, increasing amounts of DDT and DDD were deposited in the cells of the microorganisms. Two strains of lactobacilli tested were ineffective in degrading DDT (Mirna & Coretti, 1974*a*). In culture media γ-HCH and MLN were metabolised by micrococci, lactobacilli and yeasts, respectively to a rather limited degree.

The effect of enzyme systems on the degradation of DDT, γ-HCH and MLN decreased remarkably with declining pH values.

The reduction in the content of pesticides does not necessarily indicate a decrease in the hazards of environmental chemicals to the consumer. Investigations by Engst *et al.* (1974) have shown that γ-HCH is metabolised to the very persistent hexachlorobenzene (HCB) by a not clearly defined strain of mould. According to Dorough (1970) some of the metabolites of methylcarbamate insecticides may be more toxic than the original compound. Deposition of pesticides into cells and formation of conjugates also influence the extractibility; therefore, the results of pesticide analysis may be incorrect. The ability to accumulate DDT is also known in

soil microorganisms and water microflora (Kokke, 1970). The process of reductive dechlorination from DDT to DDD is frequently observed under anaerobic conditions but in aerobic media, such as sausage mixtures, this reaction is very ineffective (Walker, 1969).

A more extensive degradation of DDT and γ-HCH was observed in dry sausages with nitrite-curing salt than with starter cultures and nitrate.

Curing, hot-smoking and cooking reduced the DDT content in the final product to 61, 75 and 87 %, and the γ-HCH content to 80, 88 and 35 % of the initial amounts, respectively. The high loss of γ-HCH by cooking is due mainly to the volatility of this compound with water vapour. Similar results were obtained by Morgan *et al.* (1971) and by Ritchey *et al.* (1972) who compared the levels of γ-HCH in raw and cooked chicken tissue.

In the experiments with MLN only 2 % of the total radioactivity could be extracted after either curing, hot-smoking or cooking. According to Suess (personal communication), the radioactivity in the feeding material used consisted of more than 80 % unchanged MLN, which was extractable only after enzymic degradation by cellulase. It is assumed that mainly MLN-conjugates or non-extractable metabolites are present also in tissue of the test animals. Enzyme systems of meat tissues which are released into the sarcoplasma after freezing (8 days at −20 °C) metabolised DDT, γ-HCH and MLN to a limited extent.

In liver sausage mixtures, which are rich in enzymes, residual amounts of DDT and γ-HCH, respectively increase when sodium chloride is replaced by NCS or NCS and NaASC. This effect is more pronounced in sterilised products. By the addition of NCS, but especially of NCS and NaASC, the reactive groups of the tissue homogenate may be inhibited. According to Koransky *et al.* (1964) the degradation of γ-HCH is controlled by enzyme systems of the liver microsomes. Blocking the enzyme activity of tissues by nitrite, as reported by Warrier *et al.* (1973), might cause higher values of extractable unchanged pesticides.

The ascorbic acid oxidation system of Brodie *et al.* (1954) is used to simulate reactions occurring in biological media. As Balba & Saha (1974) have shown, the carbamate insecticide, Matacil (4-dimethylamine-3-tolyl-N-methylcarbamate) can be degraded by this system to compounds formed under field conditions. With a modified oxidation system no distinct effect with regard to an increased degradation could be observed. It may be assumed that ascorbic acid is used up by rather fast reactions in the presence of nitrite so that the residual amount of ascorbic acid becomes too small to catalyse oxidation reactions in an effective manner. Moreover, partly anaerobic conditions are present in the sausage mixture which may also inhibit the process of oxidative degradation.

The amounts of DDT and γ-HCH yielded by the extraction procedures used were not related to the manner in which contamination of the substrate occurred, whether by feeding the test animals or by adding the pesticides to the sausage mixtures. In the case of MLN, distinct differences in the results were obtained; the amount of

extractable MLN from tissue spiked by feeding was very small compared with the tests where the pesticide was added directly to the substrate.

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REFERENCES

BALBA, M. H. & SAHA, J. G. (1974). *Bull. Environ. Contam. & Toxicol.*, **11**, 193.
BRODIE, B. B., AXELFORD, J., SHORE, P. A. & UDENFRIEND, S. (1954). *J. biol. Chem.*, **208**, 741.
CORETTI, K. (1972). *Jahresbericht der Bundesanstalt für Fleischforschung*, I 48.
DOROUGH, H. W. (1970). *J. Agr. Fd Chem.*, **18**, 1015.
ENGST, R., MACHOLZ, R. M. & KUJAWA, M. (1974). *Unkonjugierte Metabolite. Nahrung*, **18**, 737.
HAMM, R. & KÖRMENDY, L. (1969). *J. Fd Sci.*, **34**, 453.
HAMM, R. & EL-BADAWI, A. A. (1972). *Z. Lebensm. Unters.-Forsch.*, **150**, 12.
KOKKE, R. (1970). *Nature*, **226**, 977.
KORANSKY, W., PORTIG, J., VOHLAND, H. W. & KLEMPAU, I. (1964). *Naumyn-Schmiedeberger's Arch. exp. Path. Pharmak.*, **247**, 49.
MIRNA, A. & CORETTI, K. (1974*a*). *Isotope Tracer Studies of Chemical Residues in Food and the Agricultural Environment.* Panel Proc. Series, IAEA, Vienna, STI/PUB/363, p. 51.
MIRNA, A. & CORETTI, K. (1974*b*). *Fleischwirts.*, **54**, 81.
MIRNA, A., HECHT, H. & CORETTI, K. (1975). *Origin and Fate of Chemical Residues in Food, Agriculture and Fisheries.* IAEA, Vienna, STI/PUB/399, p. 115.
MORGAN, K. J., ZABIK, M. E. & FUNK, K. (1971). *Poultry Sci.*, **51**, 470.
RITCHEY, S. J., YOUNG, R. W. & ESSARY, E. O. (1972). *J. Agric. Fd Chem.*, **20**, 291.
ROSSMANITH, E., MINTZLAFF, H.-J., STRENG, B., CHRIST, W. & LEISTNER, L. (1972). *Jahresbericht der Bundesanstalt für Fleischforschung*, I 47.
WALKER, C. H. (1969). *Life Sci.*, **8**, 1111.
WARRIER, S. B. K., NINJOOR, V., SAWANT, P. L., HIRLEKAR, M. G. & KUMTA, U. S. (1973). *Fleischwirts.*, **53**, 980.