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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 21 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Propanil: Propanil Task Force Response to the Reregistration Standard: Residue Chemistry Data (MRID nos. 41755001 and 41755301); Chemical No. 28201; Branch No.: 7622; DP Barcode No.: D160814

FROM: Christine L. Olinger, Chemist
Special Review Section I
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THRU: Edward Zager, Chief
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TO: Lois Rossi, Chief
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Special Review and Reregistration Division (H7508C)

Attached is the review of residue chemistry data submitted by the Propanil Task Force in response to the Propanil Guidance Document dated 12/87. This information was reviewed by Acurex Corporation under supervision of CBRS, HED. The data assessment has undergone secondary review in the branch and has been revised to reflect branch policies.

The due date for the review was 5/23/91.

The nature of the residue in poultry is now considered to be adequately understood. Additional information is required regarding the residue analytical method.

If you need additional input please advise.

Attachment 1: Review of Propanil Residue Chemistry Data

cc: (with Attachment 1): CLOlinger (CBRS), Circulation, Reg. Std. File, SF, RF, C. Furlow (PIB/FOD), Acurex

ATTACHMENT 1

PROPANIL
(Chemical Code 028201)
(CBRS No. 7622; DP Barcode D160814)

TASK 3

Registrant's Response to Residue Chemistry Data Requirements

June 20, 1991

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:

Acurex Corporation
Environmental Systems Division
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Research Triangle Park, NC 27709

PROPANIL

(Chemical Code 028201)

(CBRS No. 7622; DP Barcode D160814)

REGISTRANTS RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task-3

BACKGROUND

The Propanil Guidance Document dated 12/87 required data pertaining to the metabolism of propanil in poultry. The Guidance Document specified that the ^{14}C-balance for each analyzed sample be presented and that conjugated or bound ^{14}C-residues be characterized. Particular attention was to be given to the quantification of 3,4-dichloroaniline (DCA). In addition, representative samples from the metabolism studies were to be analyzed using all current and proposed enforcement procedures. In response to these requirements, the Propanil Task Force submitted data pertaining to the metabolism of propanil in laying hens (1990; MRIDs 41755301 and 41754401, CBRS No. 7622). These data are reviewed here for their adequacy in fulfilling outstanding residue chemistry data requirements.

The Guidance Document also required data from testing propanil using FDA Multiresidue Protocols. These data have been submitted (MRID 41755001) and have been forwarded to FDA for review and inclusion in PAM, Vol. I.

CONCLUSIONS/RECOMMENDATIONS

1. The qualitative nature of the residue in poultry is adequately understood. The data indicate that propanil is metabolized to a variety of products related to the parent chemical and 3,4-dichloroaniline (DCA). The predominant metabolites detected in hen tissues and eggs were 3',4'-dichloroacetanilide, 3,4-dichloroaniline-N-sulfamic acid, 3',4'-dichlorolactanilide, DCA, and propanil. Metabolites that accounted for >10% of the total radioactive residue (TRR) were 3',4'-dichloroacetanilide (found in eggs and all tissues except kidney tissue) and 3,4-dichloroaniline-N-sulfamic acid (found in all tissues and egg except fat). 3',4'-Dichlorolactanilide accounted for 2.40%-14.0% of the TRR in tissues and eggs. The radioactive residue accounted for by DCA ranged from 0.57% detected in skin, to 11.91% detected in eggs. No DCA was detected in thigh muscle or fat. Propanil was detected (0.52% - 11.25%) in every tissue except breast muscle.

2. A GLC method identified as the residue analytical method was tested using egg, liver, kidney, and muscle samples bearing ^{14}C-residues from the metabolism study. The

method recovered, respectively, 70 and 64% of the egg and muscle residues, but only 27 and 33% of the kidney and liver residues as 3,4-dichloroaniline (DCA).

2b. The residue method tested in this study represents a substantial modification of Method II in PAM, Vol. II. Therefore, if the registrants are proposing their GLC method as the tolerance enforcement method, it must be validated by an independent laboratory and the results submitted to the Agency for review prior to undergoing Agency validation for inclusion in PAM, Vol. II.

2c. 3,4-Dichloroaniline-N-sulfamic acid was identified as a major metabolite, accounting for approximately 23% of the liver TRR and 52.5% of the kidney TRR. This metabolite is not convertable to DCA using the registrant's GLC residue Method. If the Agency determines that 3,4-dichloroaniline-N-sulfamic acid is toxicologically significant, additional data collection and enforcement methodology will be required. This determination will be made upon completion of the ruminant metabolism study.

Note to SRRD: The current tolerance definition specifies "propanil and metabolites." Because specific compounds have been identified as components of the terminal residue in poultry, the tolerance expression will require revision. Following completion of the ruminant metabolism studies, the tolerance definition will have to be changed to include specific metabolites of concern.

DETAILED CONSIDERATIONS

Thirty White Leghorn laying hens were each dosed orally with a gelatin capsule containing 6.17 mg/day of [^{14}C-]propanil (equivalent to 51.42 ppm in the feed) for 7 days. An additional dose of 6.62 mg (55.16 ppm) was given on day 8. The daily propanil dose corresponded to 3.98 mg/kg body weight and represented 13x the theoretical exposure due to tolerance level residues in rice commodities in poultry diets (40 CFR § 186.1875). Propanil, 3',4'-dichloro-propionanilide, was uniformly labeled with ^{14}C in the aromatic ring and had a specific activity of 15.04 μCi/mg with a radiochemical purity of 98.52%. Excreta and eggs were collected daily and the samples stored frozen (-15 to -20 °C). The treated animals were sacrificed 8 hours after the last dose and blood (with EDTA added) and tissue samples were collected and frozen. All organs, tissues, excreta, egg, and blood samples were shipped on dry ice and kept at -15 °C until analysis. Solvent extracts of the samples were refrigerated until analysis by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Analyses occurred within 2 weeks after extraction.

Total Radioactive Residues (TRR)

Subsamples of tissues, excreta, egg and post extraction solids were combusted and radioassayed by liquid scintillation spectrometry (LSS). Soluble fractions from tissues were

radioassayed directly by LSS. Calculations required to determine the limit of detection of the radioassay were included but data on unfortified control samples were not included. The TRR in eggs increased through day 8, dose 7, but was lower in day 8, pm, dose 8. Egg yolk samples (1.313 ppm) showed higher TRR levels than samples of egg white (0.044 ppm). The TRR expressed as ppm in propanil equivalents in muscle tissues, fat, skin, and eggs are presented in Table 1. The data represent pooled tissues that were subsequently subsampled. Unless otherwise specified, egg samples analyzed were collected on day 8 following the eighth dose.

Table 1. Total radioactive residues (TRR; ppm propanil equivalents) found in eggs and tissues from hens dosed with ^{14}C-propanil.

Matrix	TRR (ppm)
Breast Muscle	0.230
Thigh Muscle	0.400
Liver	3.817
Kidney	3.780
Fat	2.084
Skin	1.030
Egg (Whole)	0.845[a]
Egg (Whole)	0.579[b]
Egg Yolk	1.313[a]
Egg White	0.044[a]

[a]Day 8, dose 7
[b]Day 8, pm, dose 8

Extraction

Homogenized egg samples were extracted three times with hexane:acetonitrile (ACN) (1:1, v/v) yielding hexane, ACN/H_2O, and post-extraction solid fractions. The ACN/H_2O fraction was concentrated and then partitioned with ethyl acetate (EtOAc) to yield an EtOAc fraction that was analyzed by HPLC, and an aqueous fraction. The aqueous fraction was eluted from a C_{18} Sep-Pak cartridge with methanol (MeOH), resulting in a MeOH fraction which was not analyzed further, and an aqueous fraction that was subjected to HPLC. The solid fraction was totally hydrolyzed by protease. The resulting hydrolysate was partitioned with EtOAc to yield an EtOAc fraction (not analyzed further) and an aqueous fraction. The aqueous fraction was then passed through a C_{18} cartridge, followed by MeOH elution resulting in MeOH and aqueous fractions that were not analyzed further.

A solvent combination of chloroform ($CHCl_3$):MeOH:H_2O (5:11:5, v/v/v) was used to extract residues from liver, kidney and muscle samples. After extraction, the tissue homogenates were centrifuged and the solids were re-extracted in $CHCl_3$ and centrifuged.

The supernatant solutions were combined and the layers separated to yield $CHCl_3$ and $MeOH/H_2O$ fractions. The $CHCl_3$ fraction was evaporated and the residue was taken into hexane and ACN (1:1, v/v) to yield an ACN fraction and a hexane fraction. The solid fraction was enzyme digested to yield a hydrolysate and a solid fraction. The hydrolysate was partitioned with EtOAc to yield an EtOAc fraction and an aqueous fraction. The aqueous fraction was then passed through a C_{18} cartridge, followed by MeOH elution in a similar manner to the egg sample. The remaining insoluble residues were further hydrolyzed in 1 N hydrochloric acid (for one hour) followed by an EtOAc extraction. The acid hydrolysis procedure was not applied in the fractionation of muscle samples. The ACN, $MeOH/H_2O$ and EtOAc fractions from liver and kidney samples were subjected to HPLC. The ACN and $MeOH/H_2O$ fractions from skin and breast and thigh muscle were subjected to HPLC.

A fat sample was extracted with hexane and filtered. The insoluble material was blended with MeOH and centrifuged. The MeOH soluble fraction was separated from the solids. The hexane extract was partitioned with ACN to yield a hexane soluble fraction which was not analyzed further and an ACN soluble fraction which was subjected to HPLC.

It was stated that the skin sample was processed in a similar manner as described for the fat sample, except that the initial solid fraction was further hydrolyzed with protease enzyme. From the data provided, it appears that the resulting hydrolysate was then extracted with EtOAc followed by elution through a C_{18} cartridge, in a similar manner to the egg sample. The ACN and MeOH fractions were subjected to HPLC.

Excreta samples were blended in $CHCl_3$:MeOH:H_2O (5:11:5, v/v/v) as described for the tissue extractions, resulting in $CHCl_3$, $MeOH/H_2O$, and solid fractions. The $MeOH/H_2O$ fraction was concentrated to dryness, redissolved in MeOH, filtered and analyzed by TLC.

The distribution of ^{14}C-activity in extracts of tissues and eggs is summarized in Tables 2-5.

Table 2. Distribution of radioactivity in extracts of whole egg from hens dosed with [C^{14}] propanil.[a]

Fraction	% TRR[b]	PPM
Hexane	(5.30)	(0.031)
ACN/H_2O	(86.82)	(0.503)
EtOAc	70.47[c,d]	0.408
aqueous	16.35	0.095
MeOH	4.48	0.026
aqueous	11.87[c,d]	0.069
Solids (enzyme hydrolyzed)		
hydrolysate	7.88	0.046
EtOAc	(1.40)	(0.008)
aqueous	(6.48)	(0.038)
MeOH	5.67	0.033
aqueous	0.80	0.005
Total extractables	(100.00)	(0.579)

[a]Day 8, dose 8

[b]All percent values assigned to the fractions are based upon the total radioactive residue in whole eggs and are normalized to 100%

[c]Subjected to HPLC

[d]Subjected to TLC

Table 3. Distribution of radioactivity in extracts of liver and kidney tissues from hens dosed with [C^{14}] propanil.

	Liver		Kidney	
Fraction	% TRR[a]	PPM	% TRR	PPM
$CHCl_3$	(41.41)	(1.581)	(18.40)	(0.696)
ACN	38.35[b]	1.464	17.54[b]	0.663
hexane	3.06	0.117	0.86	0.033
$MeOH/H_2O$	(27.00)[b,c]	(1.031)	(58.60)[b,c]	(2.215)
Solids (enzyme hydrolyzed)	31.58	1.205	23.00	0.869
hydrolysate	(20.07)	(0.766)	(13.00)	(0.491)
EtOAc	3.19	0.122	4.34	0.164
aqueous				
MeOH	11.71[c]	0.447	4.59	0.174
aqueous	5.17[c]	0.197	4.06[c]	0.153
solids (acid hydrolyzed)	11.51	0.439	10.01	0.378
EtOAc	(4.52)[b,c]	(0.173)	(6.22)[c]	(0.235)
HCl/H_2O	(2.90)	(0.111)	(1.83)	(0.069)
Total extractables	(95.9)	(3.66)	(98.04)	(3.71)
Unextractable	(4.09)	(0.156)	(1.96)	(0.074)

[a]All percent values assigned to the fractions are based upon the total radioactive residue in the tissues and are normalized to 100%

[b]Subjected to HPLC

[c]Subjected to TLC

Table 4. Distribution of radioactivity in extracts of breast and thigh muscle tissues from hens dosed with [C^{14}] propanil.

	Breast Muscle		Thigh Muscle	
Fraction	% TRR[a]	PPM	% TRR	PPM
$CHCl_3$	(62.96)	(0.145)	(63.68)	(0.255)
ACN	61.49[b,c]	0.141	60.74[b,c]	0.243
hexane	1.47	0.003	2.94	0.012
MeOH/H_2O	(20.49)[b,c]	(0.047)	(20.22)[b,c]	(0.081)
Solids (enzyme hydrolyzed)	16.55	0.038	16.10	0.064
hydrolysate	(13.51)	(0.031)	(12.74)	(0.051)
EtOAc	5.33	0.012	6.03	0.024
aqueous				
MeOH	2.61	0.006	4.20	0.017
aqueous	5.57	0.013	2.51	0.010
Total extractables	(96.96)	(0.222)	(96.64)	(0.387)
Unextractable	(3.04)	(0.007)	(3.36)	(0.013)

[a]All percent values assigned to the fractions are based upon the total radioactive residue in the tissues and are normalized to 100%

[b]Subjected to HPLC

[c]Subjected to TLC

Table 5. Distribution of radioactivity in extracts of fat and skin from hens dosed with [C^{14}] propanil.

	Fat		Skin	
Fraction	% TRR[a]	PPM	% TRR	PPM
Hexane	(98.94)	(2.062)	(55.60)	(0.573)
ACN	89.93[b,c]	1.874	53.28[b,c]	0.549
hexane	9.01	0.188	2.32	0.024
MeOH/H_2O	(0.93)	(0.019)	(39.26)[b,c]	(0.404)
Solids (enzyme hydrolyzed)			5.13	0.053
hydrolysate			(3.80)	0.039
EtOAc			1.02	0.011
aqueous			2.78	0.029
aqueous			1.56	0.016
MeOH			1.22	0.013
Total extractables	(99.87)	(2.081)	(98.66)	(1.016)
Unextractable	(0.13)	(0.003)	(1.33)	(0.014)

[a]All percent values assigned to the fractions are based upon the total radioactive residue in the tissues and are normalized to 100%

[b]Subjected to HPLC

[c]Subjected to TLC

Hydrolysis of residues

Subsamples of the solid fractions from egg, liver, kidney, skin, and breast and thigh muscles were treated with protease at pH 7.5 for 24 hours at 37 °C. After centrifugation, the supernatants were counted by LSS.

Characterization of residues

Fractions from subsamples of egg, liver, kidney, fat, skin and breast and thigh muscle were analyzed by reverse-phase HPLC. HPLC analysis was conducted on a system equipped with a radioactivity monitor and a UV absorbance detector at 254 nm. Samples were eluted using a 2.5 mM tetrabutylammonium phosphate in H_2O:ACN gradient.

Fractions from egg, kidney, liver, muscle, fat and skin were also analyzed by normal phase two-dimensional TLC and radiochromatography. TLC analysis was done on silica gel plates using the following solvent systems:

1. ACN:toluene, (10:90, v/v),
2. 2-propanol:ammonium hydroxide (NH_4OH, 80:20, v/v),
3. n-propanol:NH_4OH, (80:20, v/v) and
4. Acetic acid:ACN:EtOAc, (4:48:48, v/v/v).

Plates containing the nonpolar fractions were developed in solvent system #2 followed by solvent system #1. Solvent systems #2 and #4 were used to develop the plates containing the polar fractions. Solvent system #3 was used as an alternate system to #2 for comparison and verification. One-dimensional TLC using solvent systems #2 and #4 were used on fractions from excreta samples. Reference standards were spotted alongside the metabolite fractions during TLC development. Visualization of unlabeled reference standards was accomplished by exposure under short wavelength UV and treatment of the plates with iodine vapors. TLC plates were scanned for radioactivity and analyzed by direct integration from the scanner. The molecular structures of the analytical reference standards used for TLC and HPLC are illustrated in Figure 1.

Figure 1. Proposed Pathway for the Metabolism of Propanil in Laying Hens

From MRID 41754401

Figure 1. Chemical names, codes, and molecular structures of propanil and its identified and putative metabolites.

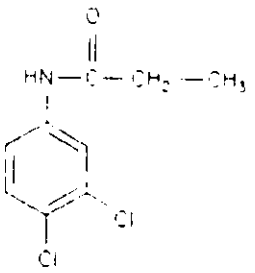
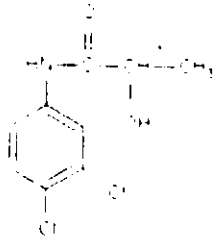
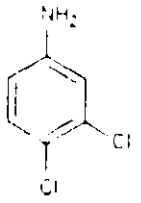
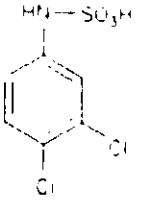
Chemical Name (Common Name)	Structure
3',4'-dichloropropion-anilide (M20)[a] (Propanil)	
3',4'-dichlorolactanilide (M13)[a]	
3,4-dichloroaniline (M18)[a]	
3,4-dichloroaniline N-sulfamic acid (M8)[a]	

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Figure 1. (continued)

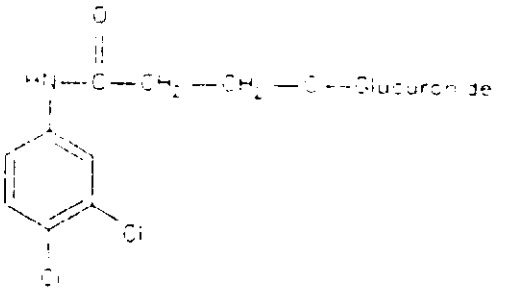
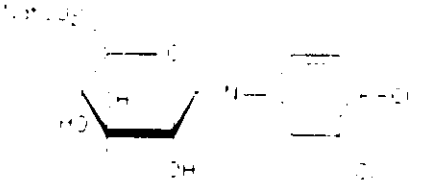
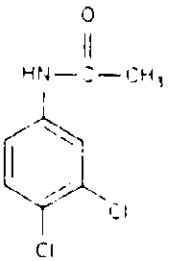
Chemical Name (Common Name)	Structure
3',4'-dichlorophenyl-propionanilide-3-hydroxy-glucuronide (M30)[a]	
N-3,4-dichlorophenyl-Na-glucuronate (M2)[a]	
3',4'-dichloroacetanilide (M15)[a]	

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Figure 1. (continued)

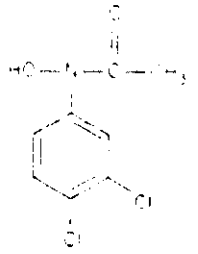
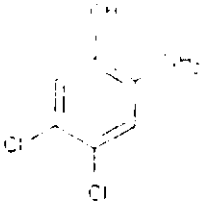
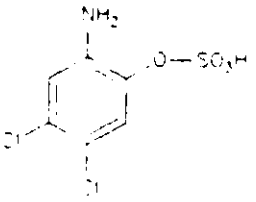
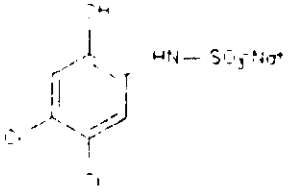
Chemical Name (Common Name)	Structure
N-hydroxy-3',4'-dichloro-acetanilide (M17)[a]	
2-amino-4,5-dichloro-phenol (M14)[a]	
4,5-dichloro-2-amino-phenol-O-sulfonic acid (M10)[a]	
4,5-dichloro-2-amino phenol-N-sulfamic acid (sodium salt) (M9)[a]	

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Figure 1. (continued)

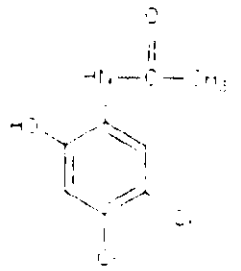
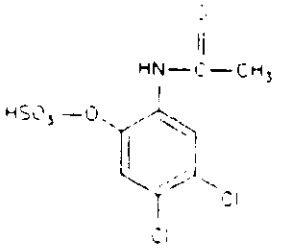
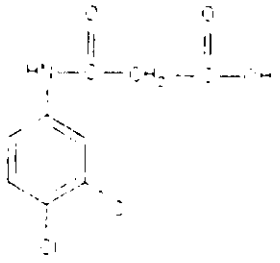
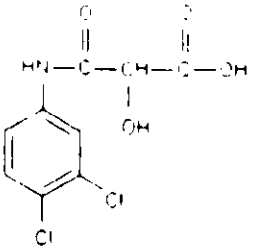
Chemical Name (Common Name)	Structure
2'-hydroxy-4',5'-dichloro-acetanilide (M21)[a]	
3',4'-dichloro-6'-O-sulfonic acid-acetanilide (M12)[a]	
3',4'-dichloromalono-anilide (M5)	
2-hydroxy-3',4'-dichloromalonoanilide (M7)[a]	

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Figure 1. (continued)

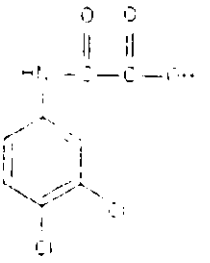
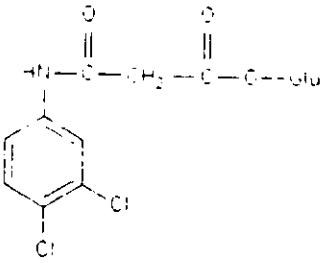
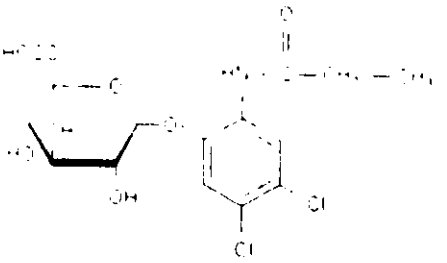
Chemical Name (Common Name)	Structure
3',4'-dichloroxaloanilide (M6)[a]	
3-carboxy glucuronide-3',4'-dichloromalonoanilide (M4)[a]	
3',4'-dichloro-6'-hydroxy-propionanilide-O-glucuronate (6-OH-propanil glucuronate) (M3)[a]	

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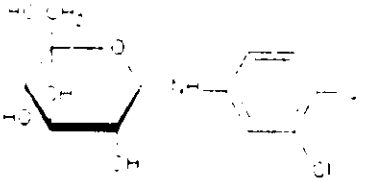
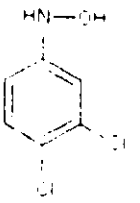
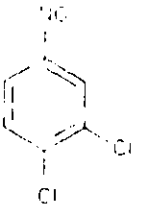
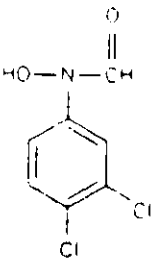
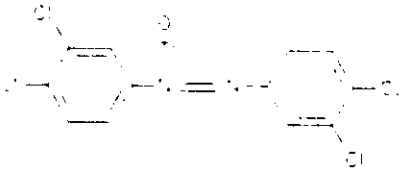
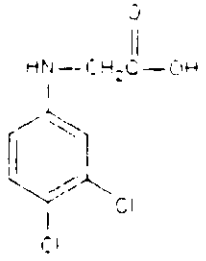
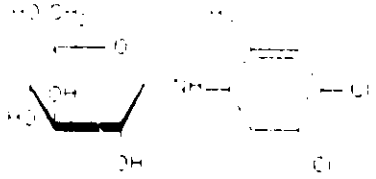
Figure 1. (continued)

Chemical Name (Common Name)	Structure
N-3,4-dichlorophenyl glucosylamine (M1)	
Free acid of M9 or M10 (M11)*	
N-hydroxy-3,4-dichloro-aniline (M19)	
3,4-dichloronitroso-benzene (M22)	
N-hydroxy-3',4'-dichloro-formanilide (M23)	

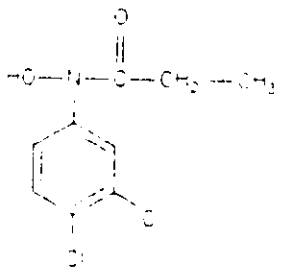
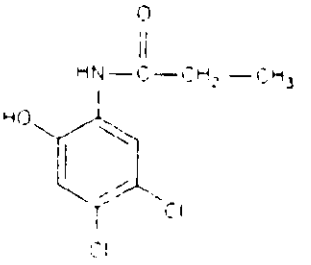
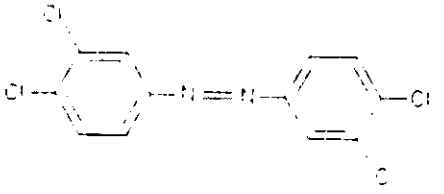
(continued)

Figure 1. (continued)

Chemical Name (Common Name)	Structure
3,4,3',4'-tetrachloro-azoxybenzene (M24)	
N-(3',4'-dichlorophenyl) glycine (M25)	
2-amino-4,5-dichloro-phenol N-glucosylamine (M26)	

(continued)

Figure 1. (continued)

Chemical Name (Common Name)	Structure
N-hydroxy-3',4'-dichloro-propionanilide (M27)	
3',4'-dichloro-6'-hydroxy-propionanilide (M28)	
3,4,3',4'-tetrachloroazo-benzene (M29)	

*Residues detected in poultry tissues and eggs (refer to Tables 6 and 7).

The major polar metabolite isolated from excreta, 3,4-dichloroaniline-N-sulfamic acid, was subjected to preparative TLC followed by HPLC purification, and then analyzed by mass spectrometry.

It was stated that certain components were unstable during TLC analysis and produced inconsistent results. Consequently, the metabolite profiles of the various extracts were established by HPLC analyses. The results from characterization of the radioactive residues in hen eggs and tissues are summarized in Tables 6-8. In liver, 95.9% of the radioactivity was extractable; 69.14% of residue was identified. The major metabolites identified in liver extracts were 3'4'-dichloroacetanilide (30.41%), and 3,4-dichloroaniline-N-sulfamic acid (22.88%). Propanil (0.52%), 3',4'-dichlorolactanilide (5.02%) and 3',4'-dichloroaniline (1.02%) were also detected.

Of the TRR in kidney samples, 78.31% was accounted for by identified metabolites. The two most abundant metabolites identified in kidney samples were 3,4-dichloroaniline-N-sulfamic acid (52.45%) and DCA (6.71%). Propanil (0.63%) and 3',4'-dichlorolactanilide (2.63%) were also detected in kidney tissue. In breast and thigh muscle, 81.98% and 80.96%, respectively, of the radioactivity was accounted for by identified metabolites. The two most abundant metabolites in muscle were 3',4'-dichloroacetanilide (52.90% in breast and 56.47% in thigh) and 3,4-dichloroaniline-N-sulfamic acid (16.51% and 17.06%, respectively). Propanil was detected in thigh muscle (1.04%), but not in breast muscle, and DCA was detected in breast muscle (1.44%), but not in thigh muscle.

The total percent of radioactive residues accounted for by identified metabolites in eggs was 81.77%. The predominant metabolites detected and identified in hen eggs were 3',4'-dichloroacetanilide (35.16%), 3',4'-dichlorolactanilide (14.00%), DCA (11.91%), and 3,4-dichloroaniline-N-sulfamic acid (11.69%). Propanil accounted for 2.68% of the TRR in eggs.

In skin and fat, 92.54% and 89.92%, respectively, of the radioactivity was accounted for by identified metabolites. The major metabolites found in skin were 3',4'-dichloroacetanilide (47.92%) and 3,4-dichloroaniline-N-sulfamic acid (31.16%). Similarly in fat, the major metabolite was 3',4'-dichloroacetanilide (71.72%). Propanil accounted for 4.17% and 11.25% of the TRR in skin and fat, respectively.

Table 6. Characterization of residues from hen liver and kidney tissues.

Metabolites	Liver		Kidney	
	% TRR	PPM	% TRR	PPM
Propanil	0.52	0.020	0.63	0.024
3',4'-dichlorolactanilide	5.02	0.192	2.63	0.099
3,4-dichloroaniline	1.02	0.039	6.71	0.25
3,4-dichloroaniline-N-sulfamic acid	22.88	0.873	52.45	1.983
Glucuronate	0.70	0.27	1.31	0.049
N-3,4-dichlorophenyl-Na-glucuronate	0.24	0.009	0.13	0.005
3',4'-dichloroacetanilide	30.41	1.161	3.47	0.131
N-hydroxy-3',4'-dichloroacetanilide	-	-	0.13	0.005
2-amino-4,5-dichlorophenol	0.84	0.032	0.75	0.028
4,5-dichloro-2-aminophenol-O-sulfonic acid	2.03	0.078	2.58	0.098
4,5-dichloro-2-aminophenol-N-sulfamic acid	2.85	0.109	4.64	0.175
2'-hydroxy-4',5'-dichloroacetanilide	-	-	0.51	0.019
3',4'-dichloro-6'-O-sulfonic acid-acetanilide	0.83	0.032	-	-
2'-OH-3',4'-dichloromalonoanilide	0.37	0.014	0.29	0.011
3',4'-dichloroxaloanilide	0.47	0.018	0.27	0.010
Carboxy propanil glucuronate	-	-	0.22	0.008
6-OH-propanil glucuronide	-	-	0.80	0.030
Free acid of M 9 or M 10	0.95	0.036	0.80	0.030
Unknown	0.62	0.024	0.13	0.005
Unknown	0.09	0.003	0.24	0.009
Unknown	-	-	0.68	0.026
Unknown	-	-	0.61	0.023
Unknown	-	-	2.37	0.090
% Identified	69.14	2.64	78.34	2.96

[a]An additional 0.121 ppm was identified in fraction EtOAc-1.
[b]An additional 0.164 ppm was identified in fraction EtOAc-1.

Table 7. Characterization of residues from hen breast and thigh muscles.

Metabolites	Breast Muscle		Thigh Muscle	
	% TRR	PPM	% TRR	PPM
Propanil	-	-	1.04	0.004
3',4'-dichlorolactanilide	7.49	0.017	5.93	0.024
3,4-dichloroaniline	1.44	0.003	-	-
3,4-dichloroaniline-N-sulfamic acid	16.51	0.038	17.06	0.068
3',4'-dichloroacetanilide	52.90	0.122	56.47	0.226
N-OH-3',4'-dichloroacetanilide	1.01	0.002	-	-
4,5-dichloro-2-aminophenol-O-sulfonic acid	1.65	0.004	-	-
Free acid of M 9 or M 10	0.98	0.002	0.46	0.002
% Identified	81.98	0.189	80.96	0.324

Table 8. Characterization of residues in skin, fat and eggs of hens administered ^{14}C-propanil.

Metabolites	Skin		Fat		Eggs[a]	
	% TRR	PPM	% TRR	PPM	% TRR	PPM
Propanil	4.17	0.043	11.25	0.234	2.68	0.016
3',4'-dichlorolactanilide	2.40	0.025	6.95	0.145	14.00	0.081
3,4-dichloroaniline	0.57	0.006	-	-	11.91	0.069
3,4-dichloroaniline-N-sulfamic acid	31.16	0.321	-	-	11.69	0.068
3',4'-dichloroacetanilide	47.92	0.494	71.72	1.495	35.16	0.204
4,5-dichloro-2-aminophenol-O-sulfonic acid	6.32	0.065	-	-	4.85	0.028
4,5-dichloro-2-aminophenol-N-sulfamic	-	-	-	-	1.48	0.009
Unknown	-	-	-	-	0.58	0.003
% Identified	92.54	0.953	89.92	1.87	81.77	0.473

[a]Whole eggs collected on day 8 following dose 8.

In summary, the qualitative nature of the residue in poultry is adequately understood. The results of the hen study indicate that propanil is metabolized to 3',4'-dichlorolactanilide and then to DCA before conjugation with acetyl and sulfate moieties. The predominant metabolites detected in hen tissues and eggs were 3',4'-dichloroacetanilide, 3,4-dichloroaniline-N-sulfamic acid, 3',4'-dichlorolactanilide, DCA and the parent compound. Metabolites that accounted for > 10% of the TRR were 3',4'-dichloroacetanilide (found in eggs and all tissues except kidney tissue) and 3,4-dichloroaniline-N-sulfamic acid (found in all tissues and egg except fat). 3',4'-dichlorolactanilide accounted for 2.40%-14.0% of the TRR in tissues and eggs. The radioactive residue accounted for by DCA ranged from 0.57%, detected in skin, to 11.91%, detected in eggs. No DCA was detected in thigh muscle or fat. Propanil was detected (0.52% - 11.25%) in every tissue except breast muscle.

The registrant has proposed a metabolic pathway which is shown in Figure 1. The primary metabolites include 3',4'-dichlorolactanilide, 3,4-dichloroaniline, and propanil. Major conjugated metabolites include 3',4'-dichloroacetanilide and 3,4-dichloroaniline-N-sulfamic acid. It appears that propanil is metabolized to 3',4'dichlorolactanilide and then to 3,4-dichloroaniline before conjugation with acetyl and sulfate moieties.

Residue Analytical Method Validation

The registrant tested their residue analytical method using samples from the hen metabolism study discussed above. The method is a based on Method II in PAM, Vol. II, with substantial modifications. As described in PAM, Vol. II, Method II is Bleidner distillation method in which residues are hydrolyzed to DCA in boiling 25% NaOH and the liberated DCA is simultaneously extracted into hexane. DCA is reacted with a color reagent and determined colorimetrically. In the Propanil Task Force method, DCA is partitioned to isooctane instead of hexane following hydrolysis in boiling 25% NaOH. The residues are cleaned up by acid-base partitioning using 3 N HCl and 25% NaOH, then partitioned into hexane and subjected to silica gel column cleanup. Residues in hexane are determined by GLC equipped with a DB-1 column using nitrogen-phosphorus detection. Recovery of radioactivity was 84-107% from eggs, 80-117% from kidney, and 95-116% from muscle control samples fortified with [^{14}C]propanil at 0.05-1.1 ppm. Recovery from liver was 94-96% at 0.6 and 1.1 ppm fortification and 141 and 127% at 0.05 and 0.11 ppm, respectively. The limit of detection of the GLC method was reported as 0.01 ppm.

Eggs (day 8, dose 8), kidney, liver, and combined thigh and breast muscle bearing ^{14}C-residues from the hen metabolism study were tested using the modified method. In addition, the isooctane extracted residues were analyzed for [^{14}C]DCA using HPLC with radiometric detection. The results are summarized in Table 9.

Table 9. Propanil residue levels in laying hen tissues and egg determined as DCA by HPLC and GLC after base hydrolysis, Bleidner distillation, and extraction.

Matrix	TRR (ppm)	%TRR in Isooctane[a]	ppm DCA HPLC/RAM[b]	ppm DCA GC/NPD[b]	ppm DCA Metabolism[c]	%TRR Not Analyzed[d]
Egg	0.579	76.3	0.442	0.408	0.373	29.6
Kidney	3.780	48.9	1.147	0.994	0.913	73.7
Liver	3.817	51.7	0.744	1.258	1.602	67.0
Breast and thigh muscle	0.315	77.1	0.243	0.201	0.200	36.2

[a]Percent TRR extracted into isooctane phase from Bleidner distillation.

[b]Found in isooctane phase from Bleidner distillation.

[c]Concentration of DCA and related metabolites found in the metabolism study which can be converted to DCA under the conditions of a Bleidner distillation.

[d]Percentage of TRR which was not determined by the GC/NPD method.

Most of the ^{14}C-residues in eggs and muscle, 70 and 64%, respectively, but only 23% of the radioactivity in kidney and 33% of that in liver were converted to DCA. The submission listed the metabolites that are likely to yield DCA following strong alkaline hydrolysis. Metabolites identified that were not included in this list are 6-OH-propanil glucuronate, 3,4-dichloroaniline-N-sulfamic acid, 4,5-dichloro-2-aminophenol-N-sulfamic acid, 4,5-dichloro-2-aminophenol-O-sulfonic acid, the free acid of either M 9 or M 10, 3',4'-dichloro-6'-O-sulfonic acid-acetanilide, 2-amino-4,5-dichloro-phenol, and N-3,4-dichlorophenyl-Na-glucuronate. These metabolites accounted for 31% of the TRR in liver and 59% of the TRR in kidney (Table 6). Of these, 3,4-dichloroaniline-N-sulfamic acid was identified as a major metabolite, accounting for approximately 23% of the liver TRR and 52.5% of the kidney TRR. If the Agency determines that 3,4-dichloroaniline-N-sulfamic acid is toxicologically significant, additional data collection and enforcement methodology will be required.

The residue method tested in this study represents a substantial modification of Method II in PAM, Vol. II. Therefore, if the registrants are proposing their GLC method as the tolerance enforcement method, it must be validated by an independent laboratory and the results submitted to the Agency for review prior to undergoing Agency validation for inclusion in PAM, Vol. II.

References:

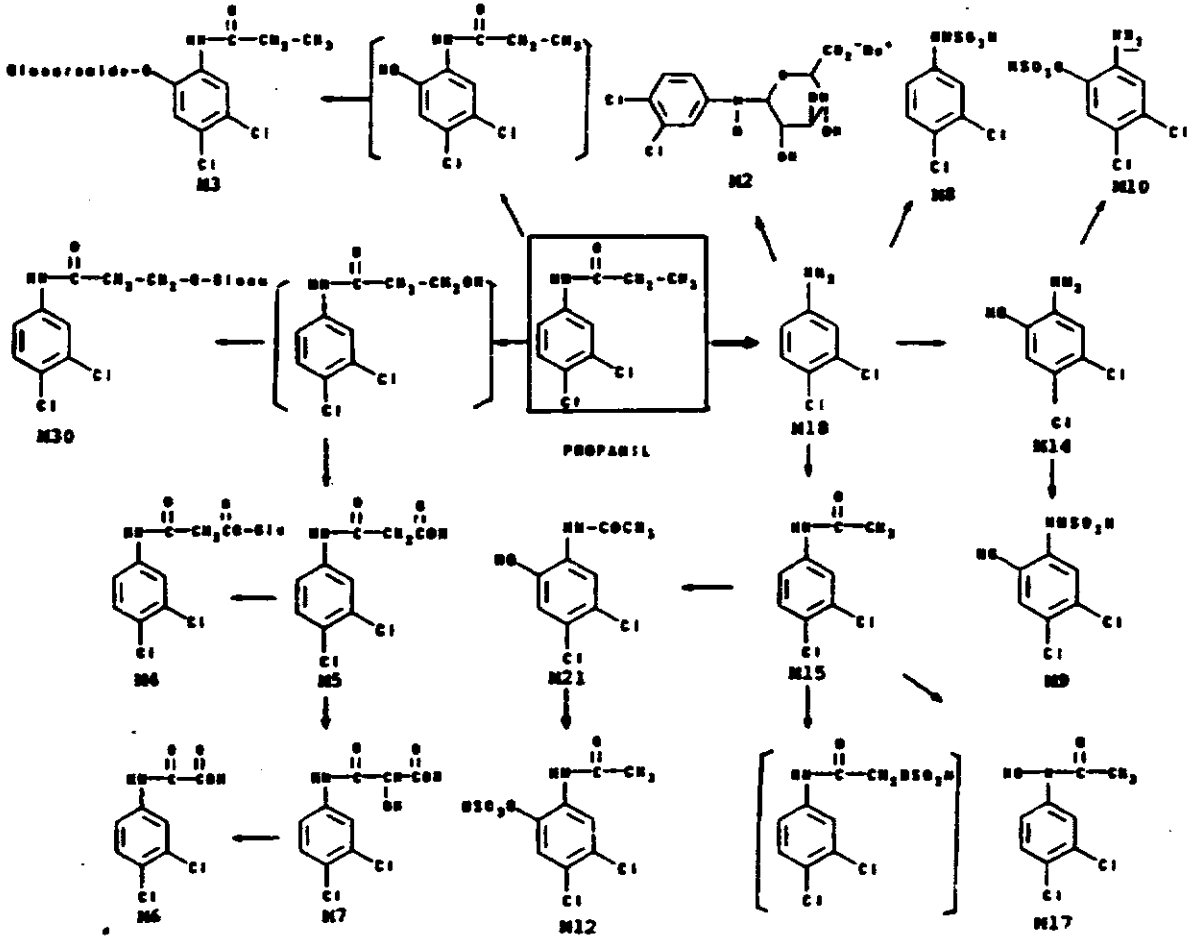
Citations for the MRID documents and Agency correspondence referred to in this review are presented below. Submissions reviewed in this document are indicated in shaded type.

41755301 Merricks, L. (1990) Metabolism Feeding Study in Laying Hens Using [Carbon 14]-Propanil In-life Phase: Lab Project Number: 2513. Unpublished study prepared by Agrisearch Incorporated and XenoBiotic Laboratories, Inc. 102 p.

41754401 Wu, J. (1990) Metabolism of [Carbon 14]-Propanil in Laying Hens -- Metabolite Analysis and Quantitation in Eggs and Tissues: Lab Project Number: RPT0028. Unpublished study prepared by XenoBiotic Laboratories, Inc. in assoc. with Agrisearch Incorporated. 124 p.

41755001 Ver Hey, M. (1989) Multiresidue Method Testing of Propanil and 3,4-Dichloroaniline: Lab Project Number: 1124. Unpublished study prepared by Colorado Analytical Research and Development Corp. 38 p.

Figure 1. Proposed Pathway for the Metabolism of Propanil in Laying Hens



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