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SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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Chlorpyrifos/059101/Dow
Non Guideline Processing Study
Processed Food and Feed – Home Cooking Studies in Various Foods

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STUDY REPORTS:

MRID No. 45619801 S. L. Byrne, G.E. Dial, S.E. Fisher, D.R. Foster, A.M. Miller, S.L. Pinkerton (25 February 2002) The Effect of Cooking on Chlorpyrifos and 3,5,6-Trichloro-2-Pyridinol Levels in Chlorpyrifos Fortified Produce; Lab Project Number: 000422. Unpublished study prepared by Dow Agrosciences 155 pages. Subsequently published as "The Effect of Cooking on Chlorpyrifos and 3, 5, 6-Trichloro-2-Pyridinol Levels in Chlorpyrifos Fortified Produce for Use in refining Dietary Exposure". S.L. Byrne and S.L. Pinkerton, *J. Agric. Food Chem.*, 52, 25 (2004) pp 7567-7573

EXECUTIVE SUMMARY:

In this non-Guideline study, chlorpyrifos (Lorsban 4E) was fortified onto several commodities (apples, broccoli, cabbage, bing cherries, green beans, peaches, bell peppers, sweet potato, acorn squash and orange juice) at a target rate of 1000 ug/g; then cooked using home preparation practices or commercial processing methods; then the effects of the processing on residues of chlorpyrifos and TCP were determined. In addition, green beans and green peppers were also grown in a greenhouse and were treated with chlorpyrifos (Lorsban 4E) at 2.2 lbs ai/A (~2X) to create naturally incurred residues, followed by the same processing treatments. Processes included baking, boiling, canning, and concentration of orange juice. Processing factors for boiling ranged from 0.32 to 1.19, with a mean of 0.75 ± 0.27. For canning factors ranged from 0.12 to 0.66, with a mean of 0.40 ± 0.20. For baking the factors that could be calculated reliably ranged from from 0.61 to 1.17, with a mean of 0.94 ± 0.27. But difficult assumptions were made in calculating the cooking factors for baked squash and sweet potato (calculating from whole RAC to separate peel and pulp), so that these latter two crops produced factors as diverse as 0.022 to 2.25]. Fourfold concentration of orange juice yielded a concentration of residue of 2.6 X. Residues incurred through green house application to the growing plants had similar processing factors to those for residues fortified directly into the harvested commodities. Where possible, TCP concentrations were measured in the cooked commodity and cooking water in an attempt to evaluate the relative effects of volatilization versus breakdown/solubility. To the same end, commodity weights were also measured before and after cooking.

Chlorpyrifos residues are not translated appreciably within plant vascular structures, but appear to be mostly surface residues. Therefore Dow considered surface treatment of commodities with chlorpyrifos to be an adequate way to produce residues. [Note that samples were peeled, sliced, etc, as in table B.1. prior to fortification.] Surface fortification was done at relatively high concentrations in an attempt to assure that measurable residues would be present after cooking,

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or at least to allow a 100 fold cooking factor. [Note that these very high concentrations also pose a serious difficulty for application of these factors to real residue data, since it is unknown whether the factors remain constant at such different concentrations.] Application to greenhouse plants was done just prior to harvest, and the rate was calculated based upon the surface area of the pot but the entire spray was directed only to the fruit of the plant. Samples were prepared for analysis by pulverization in a hammer mill under liquid nitrogen. (Water samples were not milled.) Chlorpyrifos and TCP were measured using GC with NICI MS. All analyses were performed within about 90 days of fortification and stored frozen, so storage stability data were not collected.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the processed commodity residue data are scientifically acceptable. No important scientific deficiencies were noted.

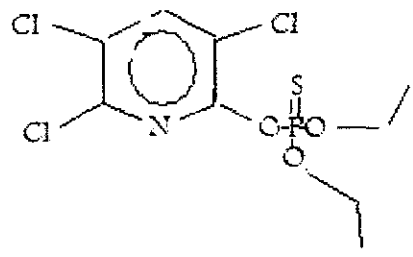
COMPLIANCE:

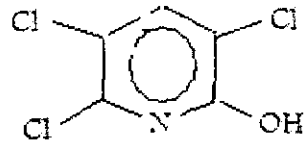
Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. Guideline studies do not allow for fortification of commodities with residues. Normally residues must be incurred. This was not a Guideline study, but with the exception of fortification of the commodities, and preparation of samples prior to fortification, there were no deviations from normal regulatory requirements that would affect the validity of the study.

A. BACKGROUND INFORMATION

Chlorpyrifos is an organothiophosphate insecticide. For the purposes of this study it is formulated into Lorsban 4E, an emulsifiable concentrate. Chlorpyrifos is used on a wide variety of crops, and while it may be applied several ways, it is frequently foliarly applied, as is the assumption of this study. Chlorpyrifos residues are generally at the surface of foods as it shows little tendency to be translocated within plants.

TABLE A.1. Test Compound Nomenclature.

Compound	Chemical Structure
	
Common name	: chlorpyrifos

Company experimental name	
IUPAC name	
CAS name	O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
CAS #	
End-use product/EP	
Compound	Chemical Structure 
Common name:	TCP
CAS name	3, 5, 6-trichloro-2-pyridinol

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

Crops were either treated by application of Lorsban 4E in a greenhouse just prior to harvest, or by direct fortification. Direct fortification was done by even application at a rate of 2 mL /Kg crop with a CAMAG TLC sprayer (Note that this is the handheld sprayer for applying derivatization reagents; it is not a Linomat spray applicator, as this reviewer originally thought.) The solution applied was 0.105 mL Lorsban 4E diluted to 100 mL in tap water, yielding a 500 ug/mL solution. After the application had dried, the treated produce was covered with a plastic film and stored in a refrigerator at ~42° F.

Application to the growing crops in the greenhouse was done for some green beans and peppers. Seeds were planted on 4 September 2001, with some additional beans planted on October 31. Plants were fertilized and treated with non-OP pesticides as needed. Target plants were treated with 2.5 kg/Ha (0.025mg/cm^3). 0.50 mL Lorsban 4E was diluted with 100 mL tap water and 5.3 mL was applied per pot. A plant to be treated was sprayed with an appropriate aliquot using a CAMAG handheld reagent sprayer. The solution was applied foliarly: onto the fruit and leaves of the plants and the solution was allowed to dry.

Water was also fortified and tested for comparison. Water was fortified as follows: 1500 g water was heated to boiling, and then a 5 mL aliquot of either chlorpyrifos or TCP was added, and boiled for 15 minutes. After cooling the water was weighed and several aliquots were taken and the remainder was discarded.

TABLE B.1.2. Study Use Pattern for the Greenhouse Treatment of Green beans and Bell Peppers				
Location	EP[1]	Application – One Application only	Tank Mix/	Harvest

		Method/Timing	Volume [2]	Rate (lb a.i./A)				
Indianapolis - Greenhouse	Lorsban 4E	1. Green beans – apply directly to fruit - 6 days PHI		2.2 lbs ai/A			None	Hand pick
		2 Green beans – apply directly to fruit - 5 days PHI						
		3. . Bell Peppers – apply directly to the fruit – 7 days PHI						

[1]EP = End-use Product

B.2. Sample Handling and Processing Procedures

Five to seven days after application, all greenhouse crops were harvested, irrespective of produce growth. Produce was randomly selected and combined into samples. Duplicate treated samples were created for processing by each cooking procedure used. The fortified samples were refrigerated for 22 – 45 hours after fortification. Samples were spread on trays, and subsamples from opposite ends of the treated trays were combined into a single sample to try to provide unbiased samples. Then both RAC and cooked samples (including orange juice) were frozen and were pulverized in an Agvise model 2001 hammer mill under liquid nitrogen, and stored frozen until analysis. Only water samples were not so milled. Water samples were stored frozen, thawed and aliquotted into subsamples.

Processing was done as boiling, baking, canning and concentration (of orange juice only). The protocol for this study was reviewed by HED prior to the initiation of the study. Conditions for boiling, baking and canning were specifically discussed between Dow and EPA, and standard cooking conditions were recommended (by Carol Lang of EPA) based upon conditions recommended in the *Joy of Cooking* (edition unspecified).

Boiling

For most crops 1500 g of tap water was brought to a boil in a saucepan 500 g of produce was added and boiled 8-20 minutes, samples were cooled and the liquid was separated from the solids. For apples and cherries, 65 g of water was added, boiling of cherries was not started until after the cherries were added, and the liquid and solids were not separated after cooking. Each phase was weighed and samples were chopped and frozen.

Baking

For most crops 500 g of sample was placed in a tared glass crystallization dish and weighed. Then the sample was cooked at 177° C (or 204° C for sweet potatoes) for 28 – 55 minutes, until soft enough to be easily pierced with a fork. The peel was separated from the pulp of the sweet potatoes and the acorn squash. Peel and pulp were weighed. Large pieces were diced and frozen until homogenization.

Canning

Sweet potatoes and acorn squash were hot packed. Respectively, 500 g and 100 g of sample were added to boiling tap water, and the mix heated just to boiling. This cooked mix was poured immediately into tared canning jars, weighed, and the jars were sealed.

Apples, cherries, green beans, and peaches were cold packed. 500 g sample was placed into the tared canning jar, weighed, the jar was filled with boiling water, weighed again, and the jar was sealed.

Both hot and cold packed jars were placed (sealed) into Presto pressure cookers containing approximately 5 cm of boiling water. The pressure cookers were covered and heat was applied until the pressure was sufficiently high to hold the vent lock in place.

Juice Concentration

Orange juice was transferred into a 1000 mL round bottom flask and concentrated under vacuum on a rotary evaporator at 80° C.

Table B.1. Cooking Procedures Applied to Specific Types of produce

Commodity	Preparation (done prior to fortification)	Boiling		Baking		Canning	
		Water Added (g)	Time (min)	Temperature (°C)	Time (min)	Pressure (psi)	Time (min)
Apple	500 g Peeled, cored, sliced	65	17	177	32	6	9
Broccoli	500 g Cut into spears	1500	8				
Cabbage	500 g washed, outer leaves removed	1500	15				
Cherry	500g washed, pitted	65	15	177	30	6	10
Green Beans	350 g washed, stems and blemishes removed	1500	18			11	25
Peaches	500 g, peeled, halved, pits removed	1500	16			6	10
Peppers	"8 halves" washed, halved, stems and seeds/membranes removed	1500	15	177	30		
Sweet Potato	500 g, peeled, cubed for boiling and canning, left whole for baking	1500	21	204	55	10	90
Winter (Acorn) Squash	500 g, seeds removed peeled and quartered for boiling, quartered for baking, peeled	1500	20	177	55	10	90

Table B.1. Cooking Procedures Applied to Specific Types of produce							
Commodity	Preparation (done prior to fortification)	Boiling		Baking		Canning	
		Water Added (g)	Time (min)	Temperature (°C)	Time (min)	Pressure (psi)	Time (min)
	and cubed for canning						

Table B.2. Summary of Sampling for Individual Produce and Cooking Procedures							
Commodity	Number of Uncooked Controls	Number of Treated or Fortified Samples	Boiled	Boiling Liquid	Baked	Canned	Concentrated
Apple	3	6	2 f	0	2 f	2 f	0
			1 c		1 c	1 c	
Broccoli	1	2	2 f	2 f	0	0	0
			1 c	1 c			
Cabbage	2	2	2 f	2 f	0	0	0
			1 c	1 c			
Cherry	3	6	2 f	0	0	0	0
			1 c				
Green Beans	4	8	2 f	2 f	0	2 f	0
			2 c	2 c		2 c	
			2 t	2 t		2 t	
Peaches	2	4	2 f	2 f	0	2 f	0
			1 c	1 c		1 c	
Peppers	2	8	2 f	2 f	2 f	0	0
			2 c	2 c	2 c		
			2 t	2 t	2 t		
Sweet Potato	3	6	2 f	2 f	2 f	2 f	0
			1 c	1 c	1 c	1 c	
Winter (Acorn) Squash	3	6	2 f	2 f	2 f	2 f	0
			1 c	1 c	1 c	1 c	
Orange Juice	1	2	0	0	0	0	2 f
							1 c
Water-chlorpyrifos	0	2	0	2	0	0	0
Water-TCP	0	2	0	2	0	0	0
TOTAL	24	54	33	31	24	21	3

f = fortified t – treated in the greenhouse as live plant c = control

B.3. Analytical Methodology

The methods used were based upon SPE cleanup and determination by GC with NICI MS determination. Specifically, for analysis of commodities, DAS Method C-1339, "Determination

of Residues of Chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol in Crops by Capillary Gas Chromatography with Negative Ion Chemical Ionization Mass spectrometry" was used. Residues in water were measured by the closely related method modified for water (again C-1339)

The crop method consisted of a single extraction of 1.5 g sample with 30 mL acetone:water (80:20) while being shaken for 30 minutes. A 1.0 mL aliquot was acidified and concentrated by loading onto a C-18 SPE column, which was then eluted with 90:10 acetonitrile:0.1 N HCl. No indication of how eluted from C-18, but then further acidified, saturated with NaCl and partitioned into 1-chlorobutane. The 1-chlorobutane was dried, (no explanation how) and an internal standard (of 13C215N-chlorpyrifos was added. Prior to analysis for TCP the TCP was silanized. The LOD and LOQ for the study were, respectively, 0.003 ng/g and 0.010 ng/g for both compounds. The overall mean recovery was 89%. Results were corrected for recoveries.

C. RESULTS AND DISCUSSION

Table C.3 provides the results for change in residues in this study. These studies were done without storage stability support; however, all samples appear to have been stored frozen for less than 90 days so no stability data are required. The cooking procedures reflect common cooking practices and the analytical methods appear to have been adequate. The results appear to be accurate for the conditions under which they were determined, and provide good estimates of the maximum processing factors possible for these processes. However, one must be very careful in applying these results to field data or monitoring results because the concentrations of applied residues were so high, were primarily surface residues, and because commodities were sliced, peeled, cored, etc before fortification. The effects of the latter preparations are uncertain. They have been shown to be similar by comparison of fortified crops to greenhouse crops for green beans and peppers, but effects have not been tested for pulpy crops such as apples. [One may ask if application of residues to the pulp surface of an apple is the same as if the pesticide were applied to the surface of the peel.] More importantly, it is unknown whether the processing factor remains constant as the concentration fortified changes over such a large range, and logic suggests that a processing factor for already washed PDP or market basket samples is not likely to be as high as the processing factor found on these unwashed commodities, especially given that residues are known to be primarily on the surface. ..

The analytical methods have been vetted on other crops, look rugged and simple, produce good chromatograms and excellent recoveries (see Table C.1.). The reported LOD and LOQ, respectively are 0.003 ng/g and 0.010 ng/g for both chlorpyrifos and TCP.

TABLE C.1. Summary of Concurrent Recoveries of chlorpyrifos and TCP.

Analyte/Matrix	Spike level (ppm)	Sample size (n)	Individual Recoveries (%)	Average Recoveries
Chlorpyrifos				
broccoli	15	2	94, 85	90
	150	1	79	79
	1500	1	83	83
Cabbage	15	2	84, 85	85
	150	1	82	82

	1500	1	84	84
Cherry	15	2	96, 80	88
	150	1	88	88
	1500	1	79	79
Green bean	15	2	96, 81	89
	150	1	81	81
	1500	1	86	86
	7500	1	105	105
Orange juice	15	2	91, 100, 72	88
	150	1	81	81
	1500	1	79	79
	7500	1	89	89
Peach	15	2	82, 87	85
	150	1	98	98
	1500	1	88	88
Pepper	15	2	96, 96	96
	150	1	79	79
	1500	1	74	74
			111	111
Water	15	2	94, 93	94
	150	1	92	92
	1500	1	93	93
Squash	15	2	92, 91	92
	150	1	75	75
	1500	1	77	77
apple	15	2	85, 86	86
	150	1	82	82
	1500	1	84	84
TCP				
broccoli	15	2	81, 77	79
	150	1	79	
	1500	1	87	
Cabbage	15	2	87. 86	87
	150	1	90	
	1500	1	88	
Cherry	15	2	91, 122	107
	150	1	87	
	1500	1	108	
Green bean	15	2	105, 91	98
	150	1	87	87
	1500	1	99	99
Orange juice	15	2	84, 86	85
	150	1	84	84
	1500	1	99	99
Peach	15	2	68, 70	69
	150	1	79	79
	1500	1	95	95
Pepper	15	2	82, 96	89
	150	1	84	84
	1500	1	85	85
Water	15	2	84, 86	85
	150	1	82	82
	1500	1	88	88
Squash	15	2	101, 146*	101
	150	1	120	120
	1500	1	112	112
apple	15	2	78, 77	78
	150	1	81	81
	1500	1	86	86
Sweet potato	15	2	92, 81	87

	150	1	85	85
	1500	1	92	92

- Outlier

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
All	Refrigerated 42 F	22 – 45 hours	None
All	Frozen	/9 – 92 days	None

Table C.3. Residue Data from Processing Study with chlorpyrifos and TCP.

RAC	Processed Commodity	Chlorpyrifos ppb		TCP ppb		Processing Factor**
		RAC*	Cooked*	RAC*	Cooked*	
apple	Boiled	1258	700	14	15	0.56
	Baked	1359	828	15	24	0.61
	Canned	878	580	15	31	0.66
broccoli	Boiled	550	514	12	ND	0.94
cabbage	Boiled	577	480	ND	ND	0.83
cherry	Boiled	539	620	ND	11	1.15
	Baked	560	649	ND	11	1.16
	Canned	664	394	ND	15	0.59
Green beans	Boiled fortified	727	456	ND	ND	0.63
	Boiled treated	4076	2374	208	68	0.58
	Canned Fortified	662	191	ND	42	0.29
	Canned Treated	2328	832	56	159	0.36
Orange Juice	Concentrated	1267	3352	ND	19	2.65
Peaches	Boiled	581	290	ND	ND	0.50
	Canned	461	237	ND	14	0.52
Peppers	Boiled fortified	676	579	ND	ND	0.86
	Boiled treated	3403	4061	22	50	1.19
	Baked fortified	640	747	ND	13	1.17
	Baked Treated	4536	3710	25	63	0.82
Sweet Potato	Boiled	526	352	18	ND	0.67
	Baked Pulp	451***	ND	ND	ND	0.022*****
	Baked Peel	451***	1014	ND	107	2.25*****
	Baked Pulp and Peel	451***	107****	ND	19	0.22*****
	Canned	596	154	25	67	0.26
Acorn Squash	Boiled	818	263	17	16	0.32
	Baked Pulp	619***	728	ND	17	1.18*****
	Baked Peel	619***	486	ND	10	0.79*****
	Canned	685	81	18	43	0.12
Water = Chlorpyrifos	Boiled	253	ND	477	606	0.04
Water = TCP	Boiled			1330	1773	

* corrected for recovery
**Processing factors reflect changes in chlorpyrifos only, TCP is not included.
***The raw sample was analyzed whole – pulp and peel together
****This is a weight averaged result, with the pulp assumed at 10 ng/g, the LOQ
*****Based upon the aforestated difficult assumptions.

Table C.5. Summary of weights measured before and after processing.

Commodity	Cooking Process	RAC weight (g)	Water weight (g)	Cooked Pulp weight (g)	Cooked Water or Peel Weight (g)	Cooked Weight/ RAC Weight
Apple	Boiled	499.3	65.2	359.0		0.72
	Baked	505.1		387.2		0.78
	Canned	498.3	274.4	652.8		1.31
Broccoli	Boiled	489.3	1491.9	504.4	1294.4	1.03
Cabbage	Boiled	499.1	1500.3	526.5	1134.8	1.07
Cherries	Boiled	505.2	65.0	505.6		1.00
	Baked	499.6		443.0		0.89
	Canned	504.8	325.2	825.3		1.63
Green beans	Boiled	1500.1	358.9	1141.4		1.02
	Boiled	1500.3	405.4	1114.8		1.16
	Canned	350.6	409.8	758.8		2.16
	Canned	354.5	409.8	746.4		2.11
Peaches	Boiled	502.1	1500.5	512.2	1056.1	1.02
	Canned	504.5	319.7	690.7		1.37
Peppers	Boiled	259.4	1500.1	242.9	1151,4	0.94
	Boiled	369.1	1500.3	375.5	1168.5	1.02
	Baked	191.3		110.8		0.58
	Baked	370.4		269.1		0.73
Sweet Potatoes	Boiled	500.2	1500.4	519.1	1142.2	1.04
	Baked	511.2		379.6	39.7	0.82
	Canned	499.7	325.1	819.3		1.65
Acorn Squash	Boiled	500.0	1500.4	523.8	1187.3	1.05
	Baked	497.0		258.4	84.7	0.69
	Canned	501.1	321.1	783.3		1.56
Orange Juice	Concentrated	415.7		100.9		0.24
Water-chlorpyrifos	Boiled		1500		1194.2	0.80
Water - TCP	Boiled		1500.2		1135.8	0.75

D. CONCLUSION

This study shows the effect of boiling, baking, and canning on the concentrations of chlorpyrifos and TCP in a variety of fruits and vegetables. Boiling and baking had a small effect, yielding factors ranging from 0.3 to 1.2. Residues of both parent and TCP in the cooking water did not change much, so there is unlikely to be significant water extraction and degradation. Thus, where residues did reduce in boiling and baking, partial volatilization of the chlorpyrifos is a more likely cause. Canning generally reduced residues even more, but also led to a concurrent increase in fruit or vegetable weight, suggesting that dilution by increasing the water content of the food is a significant cause of these reduced residues.

E. REFERENCES

F. DOCUMENT TRACKING

RDI: David L. Soderberg (4 Dec 2007); Dennis McNeilly (4 Dec 2007); Jose Morales (4 Dec 2007); C.Swartz (4 Dec 2007).
Petition Number(s):
DP Barcode(s): D282883
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