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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 08-SEP-2004

Subject: WA-040018 & WA-020021. Glyphosate: Evaluation of Proposed Rate Increase of Glyphosate to Control Cordgrass in Washington State Tide Flats Using Rodeo® and Glypro® Herbicide.

DP Number:	303997, 304000	Decision Number:	344443, 344444
PC Code:	103601	MRID Numbers:	45779601
40 CFR 180.1400		Chemical Class:	phosphono amino acid

From: J. Meghan Carroll, M.S., Industrial Hygienist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED, 7509C)

Through: P.V. Shah, Ph.D., Branch Senior Scientist
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To: Phil Errico/ James Tompkins
Registration Division (RD, 7505C)

The State of Washington Department of Agriculture submitted two Special Local Need (SLN) registrations. WA-040018 was issued to Dow AgroSciences LLC for the use of Glypro® Herbicide (EPA Reg. No 62719-324), and WA-020021 was issued to Monsano Company for the use of Rodeo® (EPA Reg. No 542-343). The registrations were issued to help control cordgrass in the Washington tidal flats under the authority of Section 24(c) of the Federal Insecticide, Fungicide and Rodenticide Act. The registrations support an increase in the application rate of Rodeo® and Glypro® Herbicide, pesticide formulations containing 53.8% glyphosate in the form of its isopropylamine salt. A tolerance of 3.0 ppm for glyphosate residues in shellfish has been established (40 CFR 180.364). The petitioner is requesting that the aerial application rate be increased from 1 to 2 gal a.i./A, and that the rate of high volume ground application be increased from 5 to 8% v/v. To determine if the increased use rates result in higher residue levels in shellfish, the petitioner proposed a Good Laboratory Practices (GLP)-compliant study using Pacific oysters and Japanese littleneck clams in 2000. HED found this protocol acceptable provided the petitioner include crustaceans (e.g. shrimp, crabs) in their study. The petitioner submitted a study on October 16, 2002 based on their 2000 proposal; however, it did not include data on crustaceans (MRID # 45779601).

CONCLUSIONS/RECOMMENDATIONS:

HED finds the proposal for an increase in the application rate of glyphosate to control cordgrass in the State of Washington inadequate. Although the 2002 residue study (MRID # 45779601) demonstrates that the proposed increase in application rate results in residues well below the established tolerance for mulluscs (e.g. oysters, clams), no data have been submitted for crustaceans. Because shellfish tolerances are based on data from molluscs and crustaceans (OPPTS 860.1400), residue data from both types of shellfish are needed to determine if an increase in the glyphosate shellfish tolerance is appropriate.

Attachment: 45779601.DER

Document Tracking

cc: J. Meghan Carroll
RDI: P.V. Shah (9/8/04); RAB1 Chemists (9/8/04)
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Rodeo: Isopropylamine glyphosate/PC Code: 103601/Nufarm Americas Inc/Company Code: 228
DACO 6.4, 7.4, 7.8/OPPTS 860.1400/OECD IIIA 8.4.3 and IIIA 8.3
Water, Fish, and Irrigated Crops - Clams and Oysters

Primary Evaluator	J. Meghan Carroll, M.S., Industrial Hygienist Registration Action Branch 1 (RAB1) Health Effects Division (HED) (7509C)	Date: 08-SEP-2004
Approved By	George F. Kramer, Ph.D., Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED) (7509C)	Date: 08-SEP-2004

STUDY REPORTS:

MRID: 45779601. Kohn, Nancy P. and Grue, Christian E. (2000). The Quantification of Tissue Residues of Glyphosate and Aminomethyl Phosphonic Acid (AMPA) in Clams and Oysters Associated with the Application of Rodeo® to Control Smooth Cordgrass (*Spartina alterniflora*). Laboratory Project No. SS-00-0001. Unpublished Study prepared by Monsanto Company. 36 pages.

EXECUTIVE SUMMARY:

The Monsanto Company submitted a magnitude of the residue study of glyphosate (Rodeo®) in/on oysters and clams. One trial was conducted at the Battelle Pacific Northwest Laboratories in Sequim, Washington in 2000 following the protocol submitted and review by HED in 2000 (Memo, W.H. Donovan, 28-FEB-2000; D2630717).

Samples of Japanese littleneck clams and Pacific oysters were placed in treatment containers that simulated their natural environment. At the beginning of the trial, the containers were filled with 5 cm of sediment spiked with 12 ppm glyphosate. Also, once a week for four weeks, glyphosate-spiked seawater was introduced to the containers during the two daily tidal inundations. The first tidal inundations started with 2 ppm glyphosate concentrated seawater which diluted down to 0.1 ppm as untreated seawater continued to fill the container. The second tidal inundation consisted of a constant supply of 0.1 ppm glyphosate spiked seawater

The glyphosate residue was quantified by high-performance liquid chromatography (HPLC) with fluorometric detection, an adequately validated method. The limit of quantitation (LOQ) was 1 ppm, and the limit of detection (LOD) was 0.3 ppm for clam and oyster tissues. Available storage stability data support the storage conditions and the sample intervals of the submitted oyster and clam residue trial. The results from the trial indicate that the maximum residue estimate is 0.4 ppm in/on oysters and clams collected 14 days after the last exposure to glyphosate-spiked seawater.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the residue data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA review of WA040021 & WA040018 (DP#: 304000).

COMPLIANCE:

Signed and dated Good Laboratory Practices (GLP), Quality Assurance and Data Confidentiality statements were provided. The minor deviations noted did not adversely affect the outcome of the data.

A. BACKGROUND INFORMATION

Glyphosate is a member of the phosphono amino acid class of chemicals. These compounds are foliar-applied herbicides that interfere with normal plant amino acid synthesis, resulting in the inhibition of nucleic acid metabolism and protein synthesis. Glyphosate blocks the activity of an enzyme, 5-enolpyruvylshikimate 3-phosphate synthase (EPSP synthase), that is involved in aromatic amino acid biosynthesis and that is produced only by green plants. Consequently, glyphosate is toxic to all green plants and essentially nontoxic to other living organisms (G.W. Ware, The Pesticide Book, 1994).

TABLE A.1. Test Compound Nomenclature

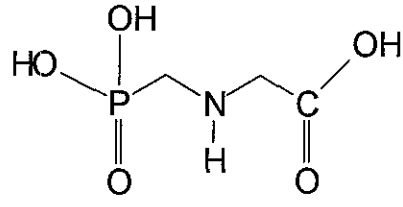
Compound	Chemical Structure
	
Common name	Glyphosate
IUPAC name	N-(phosphonomethyl)glycine
CAS name	N-(phosphonomethyl)glycine
CAS #	1071-83-6
End-use product/EP	Rodeo ®

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound		
Parameter	Value	Reference
Melting point/range	200 °C	MRID: 00161333
pH (1% solution)	2.5	www.inchem.org
Relative Density (water =1)	1.7 lb/ft^3	www.inchem.org
Water solubility (25°C)	< 0.7 g/100ml	MRID: 00161333
20% HCl solubility (25°C)	2 g/100ml	MRID: 00161333
Vapor pressure, Pa at 20 °C	Negligible	www.inchem.org
Dissociation constant (pK$_a$)	<2, 2.6, 5.6, 10.6	www.inchem.org
Octanol/water partition coefficient Log(K$_{OW}$)	-2.8	Battelle study No. SS000001, D263017

B. EXPERIMENTAL DESIGN

TABLE B.1. General Test Organism Information						
Species	Breed	Age	mean length at initiation (mm)	Weight at study initiation (kg)	Health Status*	Description of housing/holding area
Crassostrea gigas	Pacific oysters	mix	99.0 ± 3.0	NA	OK	seawater test chambers with simulated tide and photo cycles.
Tapes philippinarum	Japanese littleneck clams	mix	43.0 ± 3.0	NA	OK	seawater test chambers with simulated tide and photo cycles.

*Organisms were inspected for damage prior to trial.

TABLE B.2. Test Organism Dietary Regime		
Diet	Acclimation period	Predosing
algal slurry	10 days	none

TABLE B.3. Test Organism Dosing Regime				
Regime	Level of administered dose	Food consumption (kg/day)	Vehicle	Timing/Duration
spiked sediment	12 ppm[1]	NA	spiked sediment	1 treatment on day zero
tidal inundations of spiked seawater	0.1 ppm[2]	NA	tidal inundations of spiked seawater	8 hours wet and 4 hours dry once per week for 4 weeks.

[1] Wet sediment was mixed with an equal amount of a 12 ppm concentrate glyphosate solution and placed in the container on day zero. The sediment was not changed through the course of the experiment.
[2] On days 0, 7, 14, and 21 seawater with 2.0 ppm active ingredient was introduced to the treatment containers during the first simulated tidal inundation. As more untreated seawater entered the containers at a rate of 20 cm/hr the glyphosate concentration lowered to 0.1. During the second daily simulated tidal inundation, 0.1 ppm glyphosate seawater flowed into the dry container.

TABLE B.4. Trial Site Conditions

Trial Identification (City, State/Year)	Sediment characteristics			Meterorological Data*	Water Characterization			
	Type	%OM	pH	Overall T°C range	Type	Salinity (ppt)	pH	OM and Turbidity
Battelle Marine Science Laboratories, Bay Center, WA	loamy sand	0.8	6.8	≤ 18.0 °C	filtered seawater	27-33	7.4-8.3	NA

*The actual rainfall average and use irrigation is irrelevant to this study as it took place in a controlled treatment container in a laboratory.
Note: OM refers to Organic Material.

TABLE B.5. Commodity, Application, and Harvesting Information.

Location (City, State/Year)	EP[1]	Application					Tank Mix Adjuvants
		Treat. No.	Rate	RTI (days)	Method	Total Rate, (ppm)	
Bay Center, WA/ 2000	clams	1	12ppm	none	spiked sediment	12.2	none
		4	0.1ppm[3]	7	spiked seawater		
Bay Center, WA/ 2000	oysters	1	12ppm	none	spiked sediment	12.2	none
		4	0.1ppm[3]	7	spiked seawater		

[1] EP = End-use Product
[2] Retreatment Interval
[3] Spiked seawater was introduced at both tidal inudations on day 0, 7, 14 and 21. Two ppm spiked seawater was added to the chamber at the beginning of the first tidal inundation. It was then diluted with 20 cm/h of fresh seawater to a final concentration of 0.1ppm. The oysters and clams were again exposed to the treated seawater at the second inundation; however, this time it was the 0.1ppm diluted seawater from the first treatment of the day.

TABLE B.6. Trial Numbers and Geographical Locations

NAFTA Growing Region	Clams			Oysters		
	Submitted	Requested		Submitted	Requested	
		Canada	US		Canada	US
1						
1A						
2						
3						
4						
5						
5A						
6						
7						
7A						
8						
9						

10						
11						
12	1		1	1		1
13–21						
Total	1		1	1		1

B.2. Sample Handling and Preparation

Prior to and 24 hours after each of the four spiked seawater treatments, one oyster and four clams were collected from treatment containers. The external surfaces of the oysters and the clams were scrubbed to prevent any contaminations. The adductor muscle was sliced and the samples removed from their shells. Finally, the samples were pooled by species, frozen (< 5 °C), and shipped to the analytical laboratory.

B.3. Analytical Methodology

Glyphosate was extracted from the samples using acid and base extraction and cleaned up with Chelex 100 resin in iron form and anion -exchange column chromatography with BioRad AG 1x8 resin. The residues were identified after postcolumn derivatization with Ophthal-aldehyde (OPA) using HPLC with fluorometric detection. The glyphosate concentration was corrected based on recovery rates of spiked samples. The LOQ was 1 ppm, and the LOD was 0.3 ppm for clam and oyster tissue.

C. RESULTS AND DISCUSSION

The residues of glyphosate in Japanese littleneck clams and Pacific oysters were studied in controlled treatment containers in a laboratory. The water temperature was maintained at 16 ± 2 °C, tidal inundations were simulated, and 16 hours of light (1142 ± 52 Lux) and 8 hours of darkness were simulated inside each container. One study was preformed as specified by the HED approved protocol (Memo, W.H. Donovan, ID#000524-00343, 28-FEB-2000). Residue decline data were not submitted.

Concurrent recoveries of fortified tissue samples were within the 70-120% range suggested by OPPTS (see Table C.1). The LOQ was 1 ppm, and the LOD was 0.3 ppm for clam and oyster tissue. In two of the control samples (see Table C.3) detected residues reflected unusually high levels for tissue that had not been exposed to glyphosate. The petitioner suggests that these residues "represent an interference peak from an unknown natural substance within the tissue of the shellfish which compromised [the petitioner's] ability to detect glyphosate, even at 6 times the desired detection limits (0.05 wet weight), rather than cross contamination of the samples." Glyphosate was not detected in the sediment, control water, control bucket or algal paste. Since Rodeo® has not been used to treat Spertina within or near the clam and oyster beds that the test sample came from, the petitioner believes that the apparent residue in the control sample is unlikely to be background contamination. Further, since the fortified samples resulted in

acceptable recoveries, concentrations above 1 ppm would have been adequately quantified.

The time interval between sample collection and analysis is unknown. The worst-case estimate derived from time interval between the date of sample collection and the date the study was submitted for review is only 5 months. Based on storage stability data from fortified samples, glyphosate is a stable molecule under frozen storage conditions (-18 °C) for at least 2.5 years in a variety of matrices (Memo, C. Eiden, November 17, 1994). No corrections to the data due to storage dissipation were made.

TABLE C.1. Summary of Concurrent Recoveries of Glyphosate from Clams and Oysters.

Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev
Glyphosate				
Pacific oysters	1.0, 2.0, 3.0	1,1,1	89.0, 84.0, 92.0	88.3 ± 3.3
Japanese littleneck clams	1.0, 1.5, 2.0	1,1,1	92.0, 86.0, 90.0	89.3 ± 2.5

TABLE C.2. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration (months)*	Interval of Demonstrated Storage Stability (months)
Pacific oysters	<5 °C	5	30
Japanese littleneck clams	<5 °C	5	30

* No exact storage date was given, however the worst case estimate based on the day the samples were collected and the date the study was submitted for review is 5 months.

TABLE C.3. Residue Data from molluscs in oysters and clams with glyphosate

Trial ID (City, State, year)	Commodity Variety	Matrix	Formulation	Glyphosate Residue (ppm)							
				Day 1	Day 7*	Day 8	Day 14*	Day 15	Day 21*	Day 22	Day 35
Bay Center, WA '00	Pacific oysters	oyster tissue	glyphosate	0.3	0.3	<0.3	0.4	0.4	0.3	<0.3	0.4
			control	<0.3	---	<0.3	---	<0.3	---	0.3	<0.3
Bay Center, WA '00	Japanese littleneck clams	clam tissue	glyphosate	0.3	0.4	0.3	0.4	<0.3	0.3	0.4	<0.3
			control	<0.3	---	<0.3	---	0.4	---	<0.3	<0.3

*On these days, spiked seawater was introduced to the treatment box during the two daily tidal inundations. Also, on day 0 the spiked sediment (12 ppm) was added to the bottom of the treatment box. This sediment remained in the box throughout the trial.
— indicates that the matrix was not sampled that day.

TABLE C.4. Summary of Residue Data from molluscs with glyphosate.

Commodity/Matrix	Total Applic. Rate	PHI (days)	Residue Levels (ppm)			
			n	Min.	Max.	HAFT*
Pacific oysters	12.2 ppm	14	1	0.4	0.4	0.4
Japanese littleneck clams	12.2 ppm	14	1	<0.3	<0.3	<0.3

*Highest average field trial value

D. CONCLUSION

The residue data reflect the use of Rodeo® on the seawater and sediment in treatment containers containing Japanese little neck clams and Pacific oysters. The containers simulated the natural habitat of the clams and oysters with two daily tidal inundations, photo cycles, and temperature control. At the beginning of the trial, the containers were filled with 5 cm of sediment spiked with 12 ppm glyphosate. Also, once a week for four weeks, glyphosate spiked seawater was introduced to the containers during the two-daily tidal inundations. The first tidal inundation started with 2 ppm glyphosate concentrated seawater which diluted down to 0.1 ppm as untreated seawater continued to fill the container. The second tidal inundation consisted of a constant supply of 0.1 ppm glyphosate spiked seawater. An acceptable method was used for quantitation of residues in/on clam and oyster tissues.

E. REFERENCES

Memo, C. Eiden. Glyphosate. Residue Data on Plums, Grapes and Sugar Beets. Replacement of Craven-Data by Monsanto Submission Containing Reanalyses of Stored Samples. MRID Nos. 43315701, 43315702, 43315703, and 41940701. DP# 206278.

Memo, W. Donovan. ID# 000524-00343. Evaluation of Proposed Test Protocol to Control Cordgrass in Washington State Tide Flats Using Rodeo®. DP Barcode: D263017. Chemical # 103601. Case #:003310. Submission #:S574825.

F. DOCUMENT TRACKING

RDI: P.V. Shah (09/08/04); RAB1 Chemists (09/08/04)
Registration No. WA040018- NAF-522 and WA0400221
DP Barcode(s): 303997
PC Code: 103601

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Chemical:	**Glyphosate-isopropylammonium**
PC Code:	**103601**
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