US EPA ARCHIVE DOCUMENT

MEMORANDUM

4/17/1984

003756

SUBJECT: Review of Mutagenicity Studies on Acephate

FROM: William R. Schneider, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

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Acting Section Head, Section 2
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THRU: William Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: William H. Miller PM # 16
Registration Division (TS-767)

Acc. No. 251894, 251895 Tox. Chem. 2A

The ten mutagenicity submissions in this volume on acephate have been reviewed separately and the reviews are attached to this memo. They have also been included in the mutagenicity section of the toxicology portion of the acephate registration standard.

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Study Type: Mutagenic

Study Title: S-1248 *Salmonella* / Mammalian Microsome Mutagenicity Test with 8 Samples of Chevron Acephate Technical.

Accession No.: 251894-251895 TOX. Chem. No. 2A

Sponsor: Chevron Chemical Co., Richmond, California.

Testing Lab: same

Date: December 29, 1982

Test Materials: Eight Chevron Acephate Technicals: white powder, SX257 (85 %), SX284 (90 %), SX357 (86 %) SX911 (99.6 %), SX941 (93 %), SX976 (100 %), SX978 (98 %), SX979 (95 %).
Solvent: distilled water.

Materials and Methods:

Pour plate reverse mutation bacterial mutagenicity assays were performed according to the methods of Ames et.al., 1975*. The registrant stated that the plates were incubated for two to three days (at 37°C) and that the presence and condition of the bacterial lawn were noted. No metabolic activation was used. Solvent (distilled water) controls and positive controls were used. All eight technical batches were tested with *Salmonella typhimurium* TA-100 from 2 to 50 mg/plate except for SX976 which was tested from 0.01 to 50 mg/plate. SX284, SX911, SX976, and SX978 were also tested at 10 and 50 mg/plate with strains TA-98, and TA-1537.

Results:

The following results were reported:

S. typhimurium TA 100

Acephate (mg/plate)	SX257	SX284	SX357	SX911	SX941	SX976	SX978	SX979	H_2O
50	228	235	217	308	313	126	273	257	
20	145	201	180	214	203	121	206	190	
10	132	163	131	189	185	130	158	152	
2	122	142	123	156	145	121	137	140	
0.2						123			
0.1						120			
0.01						124			
0									120

* Ames, B., J. McCann, and E. Yanasaki. 1975, Mut. Res., 31, 347-363.

S. typhimurium TA-98

Acephate (mg/plate)	SX284	SX911	SX976	SX978
50	31	31	26	32
10	33	29	29	28
0	29	29	29	29
Positive control*	>1000	>1000	>1000	>1000

*2-Nitrofluoren (50 ug/plate)

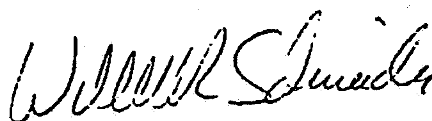
The registrant's table also listed negative results for the above compounds in another assay, but the test organism is in doubt. The table title said strain "TA 1537" but the table's heading was "TA 100".

Discussion:

This assay was obviously performed to examine the possibility that the previously obtained positive results with strain TA-100 were pecular to only that batch of acephate. This assay shows that all the batches of acephate except SX976 do produce a weak-to-moderate response at up to 50 mg/plate dosage in strain TA-100 without metabolic activation. The batch SX976 was listed as the only one which was 100% pure.

The results with strain TA-98 show no mutigenic response at 50 mg/plate with SX284, SX911, SX976, and SX978. Nothing can be said about strain TA 1537 unless the mislabling can be cleared up.

This report should be considered as supplementary information. It does raise the possibility that fully purified acephate is not mutagenic.



William R. Schneider, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

Study Type: Mutagenic

Study Title: S-1272 Salmonella / Mammalian Microsome Mutagenicity Test with 6 Samples of Chevron Acephate Technical.

Accession No.: 251894-251895 TOX. Chem. No. 2A

Sponsor: Chevron Chemical Co., Richmond, California.

Testing Lab: same

Date: December 29, 1982

Test Materials: Six Chevron Acephate Technicals: white powder, SX911 (99.6 %), SX941 (93 %), SX978 (98 %), SX984 (92.6 %), SX986 (99+ %) SX988 (96.1 %)
Solvent: distilled water.

Materials and Methods:

Pour plate reverse mutation bacterial mutagenicity assays were performed according to the methods of Ames et.al., 1975*. The registrant stated that the plates were incubated for two to three days (at 37°C) and that the presence and condition of the bacterial lawn were noted. No metabolic activation was used. Solvent (distilled water) controls and positive controls were used. All six technical batches were tested with *Salmonella typhimurium* TA-100 from 2 to 50 mg/plate except for SX986 which was tested from 0.01 to 1 mg/plate.

SX984 was SX942 (92.9%) with 0.36% acetamide added.

Results:

The following results were reported:

* Ames, B., J. McMann, and E. Yanasaki. 1975, Mut. Res., 31, 347-363.

S. typhimurium TA 100

Acephate (mg/plate)	SX911	SX941	SX978	SX984	SX986	SX988	MNNG	H_2O
50	371	306	290	257		304		
20	260	238	215	182		247		
10	212	200	168	155		184		
2	165	154	155	145		164		
1					256			
0.5					197			
0.3					151			
0.1					132			
0.0								145
(2 ug/plate positive control)							>1000	

It was stated that SX986 showed toxicity at doses above 0.5 mg/plate.

Discussion:

This experiment is not sufficient to satisfy normal requirements for an Ames test, however it obviously was designed to further examine a previously obtained Ames test. All batches produced approximately the same results: a doubling (or close to doubling) over background and a dose response relationship with the exception of SX986. This batch was stated to be 99+% pure. It did produce a dose response relationship but higher doses similar to those tested with the other batches could not be tested due to excessive toxicity.

The results with the 99+ % pure batch are pecular since in experiment S-1248, SX976 (100 % pure) produced no mutagenic response or excessive toxicity at doses up to 50 mg/plate.

This study may be considered as supplementary information. It conflicts with study S-1248 in that fully purified acephate may be mutagenic after all. All other technical batches tested were shown to be mutagenic, but weakly-to-moderately.

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CHEMICAL: Acephate

FORMULATION: Technical 93.5%

CITATION:

Mortelmans, K. E., Riccio, E. S., and G. F. Shepherd. In Vitro Detection of Mitotic Crossing-Over, Mitotic Gene Conversion, and Reverse Mutation with S. cerevisiae D7 for 7 Pesticides, EPA Contract 68-02-2947, June, 1980, SRI Project LSU-7558-20.

REVIEWED BY: William R. Schneider, Ph.D.
Toxicology Branch
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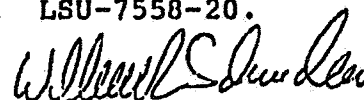

ASSAY: Iv vitro mutagenicity assay using Saccharomyces cerevisiae D7 yeast to detect mitotic crossing-over, mitotic gene conversion, and reverse mutations.

CONCLUSION:

Acephate induced mitotic crossing-over and gene conversion both with and without metabolic activation. It induced reverse mutations only in the presence of the S9 microsomal mixture.

CLASSIFICATION: Acceptable for pesticide regulation.

MATERIALS AND METHODS:

Organism: Saccharomyces cerevisiae D7

Metabolic Activation System: S9 fraction was obtained from rat liver (Arochor 1254 induced) homogenate.

Mitotic Crossing-Over Assay: The culture was incubated 4 hours in liquid suspension with the test chemical, and 10^{-3} and 10^{-5} dilutions were plated (pour plate technique) onto complete agar and scored for both mitotic recombinants (pink/red-twin sectored colonies) and other phenotypic aberrants caused by mutagenic events other than mitotic recombination. The counts are adjusted for the number of survivors.

Mitotic Gene Conversion: The treated test suspension was plated (pour plate) onto tryptophan deficient medium and scored for convertant (non-auxotrophic) colonies after incubation.

Reverse Mutation: Isoleucine deficient media in pour plate is used to score reverse mutations since the strain used is also auxotrophic for isoleucine.

Both positive and negative solvent controls were used.

RESULTS:

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Compound	MA	% Conc.	Survivors: Cells Per ml ($x 10^{-7}$)	Survivors: %	Crossing-Over: Mitotic Recombinants Per ml ($x 10^{-5}$)	Crossing-Over: Mitotic Recombinants Per 10^{-5} Survivors	Crossing-Over: Total Per ml ($x 10^{-3}$)	Crossing-Over: Aberrants Per 10^{-3} Survivors	Gene Conversion: Convertants Per ml ($x 10^{-1}$)	Gene Conversion: Convertants Per 10^{a} Survivors	Reverse Mutation: Revertants Per ml (x 10)	Reverse Mutation: Revertants Per 10 Survivors
Negative Control												
DMSO	−	10.0	5.2	100	0	0	12	23	43	8.3	22	4.3
	+	10.0	5.7	100	2.5	4.4	12	21	54	9.5	22	3.9
Positive Control												
1,2,3,4-Diepoxy-butane	−	0.013	4.6	88	97	210	810	1800	1700	370	350	76
	+	0.013	6.3	111	100	160	870	1400	1600	250	310	49
Acephate	−	1.0	3.5	67	3.0	8.6	18	51	62	18	31	8.9
	−	2.0	3.7	71	3.0	8.1	31	84	77	21	26	7.0
	−	3.0	2.7	52	8.0	30	38	140	91	34	20	7.4
	−	4.0	1.4	27	71	120	59	420	100	71	13	9.3
	−	5.0	1.3	25	20	150	53	410	83	64	4.0	3.1
	+	1.0	4.3	75	4.0	9.3	13	30	64	15	19	4.4
	+	2.0	2.9	51	3.0	10	41	140	70	24	23	7.9
	+	3.0	2.7	47	8.0	30	41	150	88	33	37	14
	+	4.0	3.4	60	18	53	57	170	110	32	37	11
	+	5.0	3.7	65	19	51	68	180	190	51	60	16

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This series of experiments was repeated with results which confirmed this pattern of mutagenicity.

DISCUSSION:

These assays were performed in a manner consistant with the most current scientific literature. The controls demonstrated the validity of the assay.

Study Type: Mutagenic

Study Title: Mutagenic Potential of Acephate Employing the L5178Y TK Mouse Lymphoma Assay. LSU-7558-21. EPA Contract No. 68-02-2947.

Accession No.: 251894-251895 TOX. Chem. No. 2A

Sponsor: US Environmental Protection Agency

Testing Lab: SRI International, Menlo Park, California

Date: September,1980

Test Material: Technical Acephate, SX734, purity not stated. White powder.
Solvent: Dimethylsulfoxide (DMSO)

Materials and Methods:

The procedures used were derived from those of Clive and Spector.* Test doses were selected by toxicity testing from 1 to 5000 ug/ml. DMSO was used as the solvent, both with and without Aroclor induced male Fischer 344 rat liver S-9 microsomal mixture. The cells were cloned in 50 ml centrifuge tubes under 5% CO_2. The cells were allowed 48 hours expression time after a 4 hour exposure period. It was not stated if the cells were exposed in the dark. Two samples per dose (8 doses from 2000 to 5000 ug/ml) were plated into a petri dish(s) for automatic plate counting after 11 days incubation at 37°C. It was not stated if more than one plate per sample was used for plate counts. A second assay without metabolic activation was performed using 10 doses from 1000 to 5000 ug/ml acephate. Ethyl Methanesulfonate (EMS) and 3-methylcholanthrene (3-MCA) were used as positive controls.

Results:

The toxicity and preliminary mutagenicity testing showed a reduction in suspension growth of 58.5% at 5000 ug/ml acephate from 100.1% at 1000 ug/ml without metabolic activation. Based on these results, a maximum dose of 5000 ug/ml was selected.

* Clive D., & J. Spector 1975. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mut. Res. 31 , 17-29.

The mutation frequency was calculated by dividing the plate counts (per ml of original culture) of the treated suspension by the plate counts of simultaneously run untreated plates. The following results were reported:

Acephate (ug/ml)	Mutant Frequencies		
	without S-9	without S-9 (repeat)	with S-9
5000	310	180	162
5000	223	167	266
4700	244	156	182
4700	250	171	203
4500	220	175	201
4500	225	184	176
4000	187	178	205
4000	188	170	183
3500	162	158	174
3500	-	149	148
3000	145	140	172
3000	132	155	170
2500	127	111	144
2500	150	106	131
2000	120	105	126
2000	110	113	170
1500		81	
1500		82	
1000		80	
1000		85	
0 (1% DMSO)	37	43	81
0 (1% DMSO)	61	54	81
500 ug/ml EMS	537	628	
500 ug/ml EMS	654	586	
5 ug/ml 3-MCA			402
5 ug/ml 3-MCA			357

Discussion:

Acephate produced a moderately positive response in this assay. Both a dose-response trend and a substancial increase (approximately half the rate of the positive controls) in mutation frequency were seen. Metabolic activation had little effect on this response. Although several details

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of the protocol were not reported (as mentioned in Materials and Methods), the consistant results and the positive findings allow us to classify this study as acceptable. It is evident from the results that the protocol was adequate.

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Toxicology Branch
Hazard Evaluation Division (TS-769)

Study Type: Mutagenic

Study Title: Mutagenesis Screening of Pesticides Using Drosophilia EPA Contract No. 68-01-2474.

Accession No.: 251894-251395 TOX. Chem. No. 2A

Sponsor: US Environmental Protection Agency

Testing Lab: WARF Institute, Inc., Madison, Wisconsin

Date: February, 1981

Test Material: Acephate, purity, batch, source, and description not stated. Solvent: 1% glucose.

Protocol: Missing from this copy of the report.

Reported Results: "Negative" at 10ppm acephate. Detailed results were missing from this copy of the report.

Study Classification: not acceptable.

Conclusions:

It was stated that acephate did not induce any sex-linked recessive lethal mutations in Drosophilia melanogaster, however we do not consider this test to be acceptable for regulatory purposes. We were not able to comment on the protocol or specific results since these pages were missing from this report and our Tox Branch copies of this report were in preprint form and did not include this information either. Even with this information, this study would be considered as supplementary information at best since the maximum amount of acephate that could be tested was 10ppm, a common problem when testing insectides in insects. The only pesticides which produced positive results were tested at 2000ppm.

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Study Type: Mutagenic

Study Title: *In vitro* Microbial Mutagenicity and Unscheduled DNA Synthesis Studies of 18 Pesticides: EPA Contract No. 68-01-2458.

Accession No.: 251894-251895 TOX. Chem. No. 2A

Sponsor: US Environmental Protection Agency

Testing Lab: SRI International, Menlo Park, California

Date: October, 1979

Test Material: Technical Acephate, SX7562, 93.5% pure.
Solvent: Either Ethanol or Dimethylsulfoxide (DMSO) (not specified)

Assays:

1. Bacterial reverse mutation plate assay in *Salmonella typhimurium* strains TA1535, TA1537 TA1538, TA98, and TA100, and *Escherichia coli* WP2.
2. Mitotic recombination induction in *Saccharomyces cerevisiae* D3
3. Differential toxicity DNA repair assays in the *E. coli* W3110/p3478 system and the *Bacillus subtilis* h17/m45 system.
4. Unscheduled DNA synthesis (UDS) in WI-38 human fibroblasts.

Conclusions: As noted in the discussion, there are problems with some of the assays.

Assay	Results: Metabolic Activation without	Results: Metabolic Activation with	Classification of data
S. typhimurium TA 1535, 1537, 1538, 98	-	-	acceptable
S. typhimurium TA 100	+ weak	+ weak	acceptable
E. coli WP2	+ weak	+ weak	acceptable
Mitotic recombination in *Sacc. cerevisiae* D3	+	+	acceptable
DNA repair differential toxicity in *E. coli* and *B. subtilis*	(-)	(-)	not acceptable
UDS in WI-38 cells	+ weak	-	acceptable

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Materials and Methods:

1. Bacterial Reverse Mutation Assay.

Bacteria: Histidine auxotrophic strains of *Salmonella typhimurium* (TA1535,TA1537,TA1538,TA98, and TA100) and the tryptophan auxotroph, *Eschericia coli* WP2 were used.

Metabolic activation system: S9 fraction was obtained from rat liver (Arochlor 1254 induced) homogenate

Salmonella assay: Different concentrations of acephate in DMSO were added to the overlay agar (including 0.05 mM histidine) and poured onto minimal agar plates, incubated 2 days at 37°C, and counted for revertant colonies. Only a single plate per dose was used.

Escherichia assay: The same techniques were used as for the Salmonella assay except that the tryptophan trace supplement was added to the base minimal agar

Controls: Both positive and solvent controls were performed with and without metabolic activation.

2. Mitotic Recombination Induction Assay.

Organism: *Saccharomyces cerevisiae* D3

Metabolic activation system: S9 rat liver fraction, as above.

Methods: Several doses of acephate were incubated at 30°C for 4 hours in suspension with the yeast cells, with and without the metabolic activation S9 mixture. Aliquots (0.2ml) of 10^{-3} dilutions are spread onto 5 agar plates, incubated for 2 days at 4°C and counted for red sectors indicative of mitotic recombination. The number of surviving cells are counted from 3 plates spread with a 0.2ml aliquote of a 10^{-5} dilution.

Controls: Both positive and solvent controls were performed.

3. Differential Toxicity DNA Repair Assays.

Organisms: *E. coli* strains W3110 and p3478
Bacillus subtilis strains H17 and M45

Methods: Several concentrations of acephate in DMSO were applied to sterile filter discs, each of which was then placed on the surface of a nutrient agar plate which had been overlayed with 2 ml of the culture in agar. The diameter of the zone of inhibition was measured after incubation at 37°C for 16 hours. Evidentally, only one plate per dose was used.

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Controls: Both positive and negative controls were used.

4. Unscheduled DNA Synthesis (UDS) Assay

Organism: Human fibroblast WI-38 cells in culture, contact inhibited, G_0 phase, preincubated 1 hour with 10^{-2} M hydroxyurea.

Metabolic activation system: S9 fraction of a Swiss-Webster mouse liver homogenate. It was not reported if the mice were treated with a liver enzyme inducing chemical.

Methods: The cells were incubated at 37° C (1 hour with or 3 hours without metabolic activation) with 1 uCi/ml of ^{3}H-thymidine (specific activity: 6.7 Ci/mmole) and several dilutions of acephate. The cells were incubated with ^{3}H-thymidine alone for an additional 3 hours following this treatment. DNA was extracted using a modified PCA-hydrolysis technique and the DNA content was measured (reference given but not described). An aliquot was scintillation counted for ^{3}H-thymidine incorporation. Six samples of each dose were tested.

Controls: Both positive and solvent controls were used.

Results:

1. Bacterial Reverse Mutation Bacterial Assay.

Acephate was tested twice with all strains of _S. typhimurium_ and four times with strain TA100. In the first experiment, the positive control was 2-Anthramine (2AA) and it worked only with metabolic activation. The second experiment used B-propiolactone for the positive control without metabolic activation and results were reported only for strains TA1535, TA1537, and TA100. The positive control results in the second experiment were reported only for strains TA1538 and TA98. The positive controls (2AA) in the 3rd and 4th experiments worked well. No significant doubling of revertants over background was seen in any of the experiments, however in the TA100 strains, a consistant dose response was seen.

Acephate was tested in 4 separate experiments with _E. coli_ WP2. Positive controls were not reported in all cases. A doubling over the background rate of revertants and a dose response were seen.

Revertants per plate: *S. typhimurium* TA100

	without S9				with S9			
Experiment #	1	2	3	4	1	2	3	4
Acephate (ug/plate)								
0	124	121	126	138	97	127	150	129
1	185				60			
10	151	109			136	150		
50	165	95			132	100		
100	154	103			130	112		
500	169	127			87	138		
1000	154	108	166		121	117	155	
2000			156				169	
2500				156				122
3000			165				166	
4000			161				172	
5000		166	172	173		177	169	155
6000				186				174
8000				165				170
10000			187	166			211	190
positive control		617			1680		588	1368

Revertants per plate: *E. coli* WP2

	without S9				with S9			
Experiment #	1	2	3	4	1	2	3	4
Acephate (ug/plate)								
0	40	27	25	24	51	20	37	46
1	44				45			
10	45				51			
50	50				58			
100	60				51			
500	38	41			46	27		
1000	59	27	34		58	30	26	
2500			25	37			38	47
5000		42	24	33		40	45	36
6000			24	49			37	45
8000			42	47			27	44
10000		52	34	38		59	48	67
positive control			158	131	720			

2. Mitotic Recombination, _Saccharomyces cerevisiae_.

No positive control results were reported however a dose response and over a ten fold increase in mitotic recombinants were seen in this study. Two experiments were performed.

Acephate (w/v)	without S9 Survivors (%)	without S9 Mitotic Recombinants (/10^5)	with S9 Survivors (%)	with S9 Mitotic Recombinants (/10^5)
		Experiment One		
0.0	100	7.5	100	7.5
0.1	119	6.3	102	1.9
0.5	108	10.5	106	12.5
1.0	96	9.8	102	7.4
5.0	89	98.0	84	84.4
		Experiment Two		
0.0	100	4.2	100	7.1
1.0	108	10.8	96	20.4
2.0	100	31.7	98	14.5
4.0	88	62.3	111	56.4
5.0	88	83.0	98	92.7

3. Differential Toxicity DNA Repair Bacterial Assays.

A 6mm zone of inhibition was reported for _E. coli_ W3110, _E. coli_ p3478, _B. subtilis_ H17, and _B. subtilis_ M45 at concentrations of 0.01, 0.1, 1.0, and 5.0 mg Acephate in 10ul DMSO on the filter disc.

4. Unscheduled DNA Synthesis in WI-38 Human Fibroblasts.

Two experiments were performed. A dose response was seen with and without metabolic activation but in the repeat experiment, a dose response was confirmed only without metabolic activation.

	dpm/ug DNA (mean of 6 samples)			
	without S9		with S9	
Experiment:	1	2	1	2
Acephate (ug/ml)				
0.0	236	135	187	81
0.1	145		145	
1.0	147		143	
10	201		170	
100	212		206	
125		126		
250		142		78
500		154		60
1000	292	183	208	76
2000		195		73
4000				74
Positive control	3238[1]	2355[1]	487[2]	325[2]

1. 4-nitroquinoline-N-oxide
2. dimethylnitrosamine

Discussion:

1. Bacterial Reverse Mutation Bacterial Assays.

Despite the lack of a doubling dose there is a dose response trend and therefore acephate may be considered to be a weak mutagen for S. typhimurium TA-100 and E. coli WP2 with and without metabolic activation. The negative findings with the other Salmonella strains are less convincing since only one plate per dose was used for counting and few positive controls were reported. In view of the fact that S. typhimurium TA100 and E. coli WP2 responded, this study is considered to be "acceptable".

2. Mitotic Recombination.

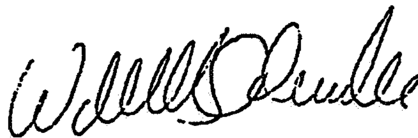
The protocol was adequate except for the lack of reported positive controls, however, since mitotic recombinants were induced by acephate, we may consider this study to be "acceptable". The study was repeated with higher doses and acephate again induced mitotic recombination and the Lowest Effective Dose (LED) was shown to be 1% acephate (w/v).

3. DNA repair in _E. coli_ and _B. subtilis_.

This study is "not acceptable" since the diameter of the zone of inhibition reported was the same as the diameter of the filter disc. This means that no zone of inhibition was seen and we can not determine if acephate diffused from the disc into the agar. There may have been no exposure of the test bacteria to the chemical.

4. Unscheduled DNA Synthesis.

A weak induction of DNA synthesis was seen without metabolic activation but not with metabolic activition. The negative control value was suspiciously high in the first unactivated experiment but the repeat experiment clearly showed a positive result and a more acceptable negative control value. The protocol was "acceptable".



William R. Schneider, Ph. D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

REGISTRATION STANDARD DER

CHEMICAL: Acephate

FORMULATION: Technical 93.5%

CITATION:

Kirkhart, B. Micronucleus Test on Acephate, EPA Contract No. 68-02-2947, March 1980, SRI Project LSU-7558-19.

REVIEWED BY: William R. Schneider, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

ASSAY: Mouse Micronucleus Test

CONCLUSION:

Acephate does not cause an increase in micronuclei when administered to mice under the conditions of this assay but the assay was not very sensitive.

CLASSIFICATION: These results may be used to supplement other chromosomal mutagenicity data.

MATERIALS AND METHODS:

Acephate (75, 150 and 300 mg/kg) in water was administered by gavage at 0 and 24 hours to 24 male Swiss mice per dose level. Eight mice were sacrificed at 48, 72 and 96 hours after first dose, bone marrow smears were made and 500 polychromatophilic erythrocytes (PCE) per mouse were examined for micronuclei. Both positive (trimethyl phosphate) (TMP) and negative controls were used.

RESULTS:

A dose related weight decrease was seen.

Acephate Dose (mg/kg)	PCE's Average with Micronuclei/500 PCE's		
	48 Hours	72 Hours	96 Hours
0	7	8	2
75	7	6	5
150	7	6	6
300	6	7	6
TMP (1000 mg/kg)	50	-	-

DISCUSSION:

The methodology used here is in agreement with the most current scientific literature. The test lacks sensitivity however, since only 500 PCE's were scored per mouse. In view of theease of scoring micronuclei at least 1000 if not 2000 PCE's should have been scored.

The negative results here would be useful in supporting the lack of chromosomal or spindle effects if an in vitro cytogenetics test were negative

REGISTRATION STANDARD DER

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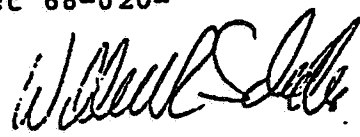
CHEMICAL: Acephate

FORMULATION: Technical

CITATION:

Evans, E. L. and A. D. Mitchell. An Evaluation of the Effects of Acephate on Sister Chromatid Exchange Frequencies in Cultured Chinese Hamster Ovary Cells. EPA Contract 68-020-2947, SRI Project LSU-7558, June, 1980.



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ASSAY: Sister Chromatid Exchange in Mammalian Cells in Culture

CONCLUSION:

Acephate causes sister chromatid exchanges in chinese hamster ovary (CHO) cells.

CLASSIFICATION: Acceptable for pesticide regulation.

MATERIALS AND METHODS:

Acephate dilutions in ethanol were added to CHO cells in flasks which were incubated for 21.5 hours, washed and treated with colchicine. Other cells were exposed to Acephate dilutions in ethanol plus Arochor 1254 induced rat liver S9 microsomal activation mixture for 2 hours. (The S9 mixture was found to be cytotoxic.) These cells were washed and incubated as above for 21.5 hurs.

At each concentration, 50 cells on slides were scored for sister chromatid exchanges.

Both positive (ethyl methanesulfonate (EMS) without activation and Dimethylnitrosamine (DMN) with metabolic activation) and negative solvent controls were used. All treatment levels were run in duplicate.

RESULTS:

WITHOUT METABOLIC ACTIVATION TREATMENT	SCE's/ CHROMOSOME	WITH METABOLIC ACTIVATION TREATMENT	SCE's/ CHROMOSOME
Negative control (0.95% ethanol)	0.803 0.537	Negative control (0.95% ethanol)	0.980 1.222
Acephate (ug/ml)		Acephate (ug/ml)	
125	0.962 0.731	312.5	0.878 0.831
250	0.881 0.731	625	0.864 0.958
500	1.253 1.189	1250	1.119 1.035
1000	1.591 1.400	2500	1.206 1.072
2000	2.605 2.710	5000	1.396 1.443
Positive Control (EMS)	1.757 2.128	Positive Control (DMN)	2.134 1.883

DISCUSSION:

Acephate was shown to produce sister chromatid exchanges in CHO cells both with and without metabolic activation. A dose response relationship was evident and, without activation, the response was greater with Acephate than with the positive control.

Study Type: Mutagenic

Study Title: Differential Toxicity Assays of Nineteen Pesticides Using *Salmonella typhimurium* Strains

Accession No.: 251894-251895 TOX. Chem. No. 2A

Sponsor: EPA Research Triangle Park Contract No. 68-02-2947

Testing Lab: SRI Int. Menlo Park, California 94025

Date: February 1981

Test Material: Acephate Technical: white powder, SX734
93.5% pure
Solvent: DMSO

Materials and Methods:

The following DNA repair deficient strains of bacteria: *Salmonella typhimurium* SL4700, and *S. typhimurium* TA 1538, were matched with repair competent strains: *S. typhimurium* SL4525, and *S. typhimurium* TA 1978. Duplicate plates for each dose were prepared by placing a 6mm filter disc inoculated with the test substance in the center of a pour plate with the top agar seeded with the test strain of bacteria. The zone of inhibition was measured including the diameter of the filter disc. A negative control, Kanamycin, was also tested and should result in equal inhibition of both strains. Positive controls were Methylmethanesulfonate for strains SL4525/SL4700 and 4-Nitroquinoline-l-oxide for strains TA1978/TA1535.

Results:

The following results were reported:

Test Substance	Dose	Average Zone of Inhibition (mm) *S. typhimurium*			
		SL4525	SL4700	TA1978	TA1538
Kanamycin	100 ug	15.5	15.0	14.5	15.5
Positive Control	10 mg	53.0	70.0	12.0	24.5
Acephate	1 mg	6.0	6.0	6.0	6.0
	5 mg	9.0	10.0	7.0	7.0

Discussion:

This experiment was conducted well and used duplicate plates which showed consistant results (identical results in the case of acephate). Positive and negative controls produced the expected results. No zone of inhibition was seen for Acephate at 1 mg dose. Since we can not determine if any diffused into the agar this is regarded as a "No-test". At 5 mg on the disc, a very small zone of inhibition was seen that was similar in both the repair deficient and competent strains. For these strains, at 5 mg dosage, acephate does not produce DNA damage as indicated by differential toxicity in repair deficient bacteria. The study may be classified as adequate for regulatory purposes.

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003756

Study Type: Mutagenic

Study Title: Further Mutagenicity Studies on Pesticides in Bacterial Reversion Assay Systems

Accession No.: 251894-251895 TOX. Chem. No. 2A

Authors: M. Moriya, T. Ohta, K. Watanabe, T. Miyazawa, K. Kato, and Y. Shirasu.

Reference: Mutation Research, 116 (1983) 185-216

Test Material: Acephate; source, batch, and purity not identified.

Materials and Methods:

Bacterial reverse mutation pour plate assays were performed according to the methods of Ames et.al. (1975). A tryptophan auxotroph, Escherichia coli WP2 hcr, was also tested in a similar procedure using tryptophan instead of histidine and biotin in the top agar. The number of plates per dose were not reported. The plates were counted after two days incubation. Numerical results for acephate were not stated however, judging from the graph, it was tested at six doses ranging from 0 to 50000 ug/plate, both with and without S9 activation mixture. The source and preparation of the S9 mixture was not stated.

Results:

It was stated that Acephate was positive with E. coli WP2 and S. typhimurium TA 100 and negative with S. typhimurium strains: TA1535, TA 1537, TA1538, and TA98. It was also stated that the presence or absence of S9 had little effect on the results with acephate. Quantitative results were not given for E. coli however a small, poor resolution graph was presented for S. typhimurium TA 100 which indicated that little increase was seen over background rate up to a dose of 5000 ug/plate whereas a strong dose response was seen in three doses from 5000 to 50000 ug/plate although there was just slighly more than a doubling of the plate count over background. The authors reported their calculation of revertants/nmole to be 0.0013.

Discussion

This paper reports the results of testing of 228 pesticides. As a result, the methodology and results are abbreviated and lack sufficient information for us to fully evaluated the study. Acephate was reported to induce reverse mutations in E. coli WP2 hcr and in S. typhimurium TA100. The graph and revertant/nmole calculation indicate that the mutagenicity in S. typhimurium TA100 is weak. No quantification of the mutagenicity for E. coli was reported. It is also impossible to evaluate the negative findings for the other Salmonella strains due to lack of information. This study must be classified as supplementary.

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