11-18-85

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

004794

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: EPA ID Number: 061602; EPA Registration No. 239-2460.
Paraquat: Evaluation of six mutagenicity studies.

Accession No. (Not assigned) Tox. Chem. No. 634
Record No. 159828 Project No. 734

FROM: Krystyna K. Locke, Toxicologist
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TO: Geraldine Werdig/Barbara Briscoe
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THRU: Edwin R. Budd, Section Head
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The following studies were reviewed by Irving Mauer, Geneticist, Toxicology Branch/HED.

1. Gene Mutation: Paraquat Dichloride (Technical Liquor): Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells, P. Clay and M. Thomas, September 24, 1985, Report No. CTL/P/1398, Imperial Chemical Industries PLC.

2. Paraquat Dichloride: Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells, M. Cross, September 17, 1985, Report No. CTL/P/1374, Imperial Chemical Industries PLC.

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3. Cytogenetics: Paraquat Dichloride: A Cytogenetics Study in Human Lymphocytes In Vitro, T. Sheldon, C.A. Howard, J. Wildgoose, C.R. Richardson, September 3, 1985, Report No. CTL/P/1351, Imperial Chemical Industries PLC.

4. DNA Damage/Repair: Paraquat Dichloride: Assessment for the Induction of Unscheduled DNA Synthesis In Primary Rat Hepatocyte Cultures, R.W. Trueman, J. Ashby, B. Burlinson, September 4, 1985, Report No. CTL/P/1339, Imperial Chemical Industries PLC.

5. An evaluation of Paraquat Dichloride (Technical) In the Mouse Micronucleus Test, T. Sheldon, C. R. Richardson, J. Shaw, G. Barber, September 4, 1985, Report No. CTL/P/1369. Imperial Chemical Industries PLC.

6. Paraquat Dichloride: An In Vitro Sister Chromatid Exchange Study in Chinese Hamster Lung Fibroblasts, C.A. Howard, J. Wildgoose, P. Clay, C. R. Richardson, September 24, 1985, Report No. CTL/P/1392, Imperial Chemicals Industries PLC.

All studies but one (# 5) were classified by I. Mauer as Acceptable. Study # 5 was classified as Unacceptable.

Paraquat was positive for chromosome damage at cytotoxic concentrations (Study # 3) and for sister chromoatid exchange at nontoxic concentrations (Study # 6), but was negative for unscheduled DNA symthesis (Study # 4).

Paraquat was weakly positive in study # 1. In that study, paraquat was not mutagenic to mouse lymphoma cells in the absence of metabolic activation (S 9 fraction from rat liver). However, there were dose-related increases in mutant frequencies in the S9-activated cultures but were regarded as "artefactual" by the investigators.

Paraquat was weakly positive in study # 2. In that study, statistically significant increases in mutant colonies were oberved at doses below 29% cell survival. However, these effects were not considered by the investigators as mutagenic "because effects observed are associated with high cytotoxicity and show threshold level".

cc: Robert Taylor (RD)

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TOXICOLOGY BRANCH DATA REVIEW

Caswell: 634
EPA Chem. 061602

CHEMICAL: Paraquat Dichloride

STUDY TYPE: Mutagenicity - Gene Mutation in Mammalian Cells (L5178Y-TK)

CITATION: Paraquat Dichloride (Technical Liquor): Assessment of Mutation Potertial Using L5178Y Mouse Lymphoma Cells

ACCESSION No./MRID No.: (Not yet assigned)/na ["REF. 1" of Submission]

SPONSOR/TESTING LAB: Chevron/ICI Central Toxicology Lab

STUDY No./DATE: ICI Report No. CTL/P/1398, 9/24/85

TEST MATERIAL: Paraquat dichloride (technical liquor), 45.66% ai, a brown liquid, dissolved in physiological saline for testing.

PROCEDURES:

Mouse lymphoma L5178Y cells of the -3.7.2c subclone (TK+/-) were exposed for 2 hours to the test material at concentrations ranging from 31.25 to 1000 ug/ml (the highest concentration severely cytotoxic), both in the absence and presence of a metabolic activation system consisting of Aroclor 1254-stimulated rat hepatic microsomes (S-9) plus appropriate co-factors. Five separate experiments were performed. Ethylmethone sulfonate (EMS), a direct-acting mutagen and benzo(a)pyrene (B(a)P), which requires metabolic activation served as positive controls.

All cell cultures were then incubted in fresh medium for 48 hours (expression time), following which they were exposed (in multi-well plates) to trifluorothymidine (TFT) which selectively kills all but TK-/-cells, i.e., newly-induced mutants. Mutation frequencies (mf per 10^{-4} survivors) for each treatment group were calculated by logit regression (dependent on the number of empty wells), which provides maximum liklihood estimates; tests for trend in log mutant frequencies (one-sided) were also calculated separately for each experiment, as well as for conbined dates across all experiments.

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Results:

Dose-related cytotoxicity to paraquat was observed in all experiments; concentrations of 500 and 1000 ug/ml were lethal in two experiments (see tabulation of combined results).

In four experiments, no significant increases were found without activation; in EXPT. 2, an increase of 2.5X solvent control was induced. In the presence of S-9, increases in mf 2 or more times control were found in 3 of the 5 experiments. These apparently positive results were ascribed by the authors to low negative control plating efficiencies and/or low cell survival. A decreasing trend in both solvent control and B(a)P mf is evident from the data reported (see tabulation), respectively, from 7.4×10^{-4} in EXPT.1 to 0.6×10^{-4} in EXPT.5, and from 20.1×10^{-4} to 2.6×10^{-4} in EXPT.4; the exception to this trend in response to B(a)P was in EXPT.5, in which a mf of 5.6×10^{-4} was calculated.

Statistical analysis of the data supported the overall conclusion on inspection that, (1) there were no treatment related effects of Paraquat in non-activated cultures; and (2) there was an overall statistically significant trend in mf with dose, as well as individual trends in EXPT.'s 1, 2 and "possibly" in EXPT. 5 [but detailed analyses for the latter were not presented - see below].

Conclusion:

The authors concluded that the test substance was non-mutagenic to mouse lymphoma cells selected in TFT. The dose-related increase in mf in activated cultures was considered to be "artefactual" (without biological significance) because of the following:

1. The effects observed appeared to be S9 dependent. Referenced articles (listed on page 10 of the report) provide "no biochemical evidence to suggest paraquat dichloride can be metabolized .... and the effects in the presence of S9 should therefore be no different from the clearly negative result observed in the absence of S9".

2. The "considerable" variation in control mf and plating efficiencies between experiments signified erratic test reproducibility (evident by the results obtained in EXPT.'s 1, 2 and 5), and may involve little-understood complex biological interactions of non-genetic orgin.

3. Thus, to the authors, the premise underlying the statistical analysis of the results --- that the only effect of high toxicity and low plating efficiency is reduction in the number of viable cells sampled (and hence an increased variance)--- "....may be unfounded." Other factors (unrelated to mutagenesis, in [2] above) "....may perturb the assay and complicate the interpretation of results."

4. On the other hand, the consistent (and positive) results obtained with B(a)P (when increases in each experiment were compared with their respective solvent controls) indicated to the authors "....that the assay is capable of reproducibly detecting a known mutagen and lends further weight to the argument that the small and erratic positive test responses caused by paraquat dichloride (technical liquor) are artefactual."

TB Evaluation:

This study is ACCEPTABLE as a comprehensive assay of paraquat dichloride in the mouse lymphoma (L5178Y) cell system. The data demonstrate a small but statistically significant increase in the frequency of mutants (by trend analysis), but only in activated (S9-supplemented) cultures. It is argued by the authors of the report that this increase ma be due to the low plating efficiency associated with low cell survivals, and may involve compound effects other than single gene mutation at the TK locus.

As indicated by one of the principal investigators who developed this assay (Clive et al., Mutation Res. 59:61, 1979; Mutation Res. 115:225, 1983 -the Gene-Tox Report; neither of these articles referenced here), this arguement can be substantiated if colony size is taken into account. Clive and colleagues showed a bimodal distribution of responses with a series of known carcinogens requiring MA, and that only the production of large colonies were "true" mutations at the TK-locus, smaller colonies representing chromosome damage to a group of loci involving both TK and adjacent genes (Hozier et al., Mutation Res. 84: 167, 1981). Elsewhere in this submission, ICI presents evidence of the in vitro cytogenetic activity of paraquat in both human lymphocytes (Report No. 1351) and DON cells (Report No. 1392). Thus, it would be more helpful in interpreting the results of this mouse lymphoma assay were both the range of background data as well as colony size distributions (with appropriate cytogenetic analysis) supplied.

PARAQUAT DICHLORIDE (TECHNICAL LIQUOR):
ASSESSMENT OF MUTAGENIC POTENTIAL USING L5178Y
MOUSE LYMPHOMA CELLS (1)

DOSE (ug/ml)	EXPERIMENT 1		2		3		4		5	
	Surv. (%)	MF ($X10^{-4}$)	SURV. (%)	MF $X10^{-4}$	Surv. (%)	MF $X10^{-4}$	Surv. (%)	MF $X10^{-4}$	Surv. (%)	MF $X10^{-4}$
DIRECT (-S9)										
EMS : 1000	15	83.3*	35	33.4*	8	22.8*	38	9.5*	16	9.1*
Solvent (Saline)	100	32.3	100	7.5	100	2.2	100	1.3	100	1.3
PARA: 31.25	122	5.0	NT		65	1.0	52	1.3	84	1.2
62.5	65	8.4	60	5.8	49	1.3	80	1.9	77	0.8
125	92	5.6	35	6.8	43	1.8	63	2.4	56	0.9
250	25	10.2	12	18.6*	6	3.9	58	1.5	26	2.0
500	21	18.2*	0	-	0	-	63	2.2	3	2.3
1000	NT		0	-	NT		NT		NT	
INDIRECT (+S9)										
B(a)P: 6	9	20.1	0	14.2*	2	7.4*	61	2.6*	5	5.6*
Solvent (Saline)	100	7.4	100	4.6	100	1.9	100	1.1	100	0.6
PARA: 31.25	62	5.3	NT		134	2.1	61	1.2	82	1.2
62.5	58	9.6*	44	9.9*	83	1.7	59	1.7	57	0.8
125	52	11.9	24	14.5*	60	1.6	72	0.8	30	1.5*
250	18	15.8*	3	45.1*	7	2.6	65	1.1	15	1.2
500	2	-	0	-	2	-	26	1.6	5	2.1*
1000	NT		0	-	NT		NT		NT	

(1) Extracted and compiled by the reviewer from Tables 1-5 of the Report.
* Increase in MF greater than two-fold the solvent control.
LEGEND: EMS, - ethylmethanesulfonate, B(a)P, benzo(a)pyrene;
PARA, - paraquat dichloride; NT, not tested;
SURV (%) - percent cell survival on plates (compared to concurrent solvent control = 100);
MF ($X10^{-4}$) - mutant frequency per 10^4 survivors.

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TOXICOLOGY BRANCH DATA REVIEW

Caswell: 634
EPA Chem. 061602

CHEMICAL: Paraquat Dichloride

STUDY TYPE: Mutagenicity - Gene Mutation in Mammalian Cells (L5178Y-TK)

CITATION: Paraquat Dichloride: Assessment of Mutation Potential Using L5178Y Mouse Lymphoma Cells

ACCESSION No./MRID No.: (Not yet assigned)/na ["REF. 2" of Submission]

SPONSOR/TESTING LAB: Chevron/ICI Central Toxicology Lab

STUDY No./DATE: ICI Report No. CTL/P/1374, 9/17/85

TEST MATERIAL: Analytical grade paraquat dichloride, a white solid 99.6% ai, dissolved in either DMSO (one experiment) or saline (5 experiments) for assay.

PROCEDURES:

Mouse lymphoma L5178Y cells of the -3.7.2c subclone (TK+/-) were exposed for 2 hours to a wide range of test material concentrations in a series of 6 separate experiments, viz., 7.87 to 1004 ug/ml, both in the absence and presence of a metabolic activation system (MA) consisting of Aroclor 1254--stimulated rat hepatic microsomes (S-9) plus appropriate co-factors. Ethylmethanesulfonate (EMS), a direct-acting mutagen, and dimethylnitrosamine (DMN) as well as benzo(a)pyrene (B(a)P), both of which requires metabolic activation, served as positive controls.

All cell cultures were then incubated in fresh medium for 48 or 72 hours (expression time), following which they were exposed (in multi-well plates) to trifluorothymidine (TFT) which selectively kills all but TK-/-cells, i.e., newly-induced mutants. Mutation frequencies (mf per 10^{-4} survivors) for each treatment group were calculated by logit regression (dependent on the number of empty wells), which provides maximum liklihood estimates.

Results:

Paraquat was cytotoxic in a dose-related fashion, more so in the presence of S9 (see attached tabulation).

The authors reported that at cell survivals of 29% or greater, the test material did not increase mf over respective solvent controls when assessed by cell growth in TFT after either 48 hours (3 experiments) or 72 hours (3 experiments). At exposure levels "....inducing excess cell death, i.e., less than 29% survival....", however, "....sporadic increases in mutation frequency [were] observed," both in the presence and absence of S9 MA, notably in EXPTs 1, 2, 4 and 6 (see summary Table attached).

Conclusions:

The authors concluded that [analytical grade] paraquat dichloride was non-mutagenic in this assay. The increase observed at levels below 29% survival were ascribed as being either: (1) "...associated with high toxicity and show a threshold level;" and/or, (2) "...induced by chromosomal rearrangements in some cells which are more competent to resist the cytotoxicity of TFT as the selective agent." [Reference is made to a positive in vitro chromosome aberration assay in human lymphocytes, CTL Report No. 1351, and a positive SCE test, CTL Report No. 1392.]

TB Evaluation:

This study is acceptable as a comprehensive assay of the test substance in the mouse lymphoma assay, but the authors' interpretation of results viz, that the positive effects found at cytotoxic doses are cytogenetic in origin rather than specifically mutational at the TK-locus would be more conclusively established were cytogenetic analysis performed. [See review of Report 1398 for background rationale.]

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PARAQUAT DICHLORIDE: ASSESSMENT OF MUTAGENIC POTENTIAL USING L5178Y MOUSE LYMPHOMA CELLS (1)

Treatment	48 HOUR EXPRESSION (1)					
	EXPT. 1		EXPT. 3		EXPT. 4	
	Surv (%)	MF ($\times 10^{-4}$)	Surv (%)	MF ($\times 10^{-4}$)	Surv (%)	MF ($\times 10^{-4}$)
DIRECT (-S9)						
EMS: 1000	9	20.5*	33	23.3*	3	24.3*
Solvent	100	2.7	100	1.7	100	1.6
PARA:						
15.7			115	0.8		
31.3 (.5)			74	0.6		
62.5(.8,.9)	37	2.5	89	1.1	26	0.9
125 (126)	18	4.5	53	1.6	38	2.1
250 (251)	4	6.4*			11	7.3*
500 (502)	2	-			1	-
1004	0	-				
INDIRECT (+S9)						
B(a)P: 6	0	15.4*	35	2.9*	0	11.2*
Solvent	100	4.3	100	1.6	100	1.7
PARA:						
7.87			106	1.6		
15.7			89	2.2		
31.3(.5)			97	1.1	78	1.6
62.5(.8,.9)	55	4.2	92	2.1	59	2.2
125 (126)	8	3.1			14*	2.3
250 (251)	0	3.8			6	11.0*
502	0	-				
1004	0	-				

(1) Extracted and compiled by the reviewer from Tables 1, 3 and 4 of the Report.

LEGEND: EMS, - ethylmethanesulfonate, B(a)P, benzo(a)pyrene;
PARA, - paraquat dichloride;
SURV (%) - percent cell survival on plates (compared to concurrent control = 100);
MF - mutant frequency per 10^4 survivors.

* Increase in MF greater than two-fold the solvent control.

PARAQUAT DICHLORIDE: ASSESSMENT OF MUTAGENIC POTENTIAL USING L5178Y MOUSE LYMPHOMA CELLS (1)

Treatment	72 HOUR EXPRESSION (1)								
	EXPT. 2			EXPT. 5			EXPT. 6		
	Dose	Surv	MF	Dose	Surv	MF	Dose	Surv	MF
DIRECT (-S9)									
EMS (ug/ml)	1000	11	46.7*	1000	43	12.1*	750	73	12.4*
							1000	47	19.8*
Saline	0	100	5.7	0	100	2.5	0	100	1.3
Paraquat Dichloride (ug/ml)	31.5	33	6.2	23.3	82	1.1	20.9	100	2.1
	62.9	19	7.3	46.5	84	2.1	41.9	82	3.2
	126	5	16.2*	93	53	1.2	83.7	61	1.7
	252	0	-	186	13	3.9	167	11	5.4*
				372	0	-	335	0	-
INDIRECT (+S9)									
B(a)P (ug/ml)	6	15	22.4*	6	4	6.8*	4	97	5.7*
							5	34	(LOST)
							6	8	8.4*
DMN (ug/ml)							0.6	43	22.7*
							0.9	37	42.8*
							1.2	23	36.4*
Saline	0	100	7.7	0	100	2.1	0	100	2.5
Paraquat Dichloride (ug/ml)	31.5	27	7.4	11.6	100	1.9	10.5	130	2.2
	62.9	7	7.7	23.3	89	1.0	20.9	71	2.9
	126	0	21.0*	46.5	89	1.8	41.9	80	2.3
	252	0	-	93	58	2.2	83.7	29	7.6*
				186	9	2.3	167	2	-

(1) Extracted and compiled by the reviewer from Tables 2, 5 and 6 of the Report.

LEGEND: EMS, - ethylmethanesulfonate, B(a)P, benzo(a)pyrene;
PARA, - paraquat dichloride; NT, not tested;
SURV - percent cell survival on plates (compared to concurrent control = 100);
MF - mutant frequency per 10^4 survivors.

* Increase in MF greater than two-fold the solvent control.

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TOXICOLOGY BRANCH DATA REVIEW

Caswell: 634
EPA Chem. 061602

CHEMICAL: Paraquat Dichloride

STUDY TYPE: Mutagenicity - Chromosome Aberrations in vitro (human lymphocytes)

CITATION: Paraquat Dichloride: A Cytogenetic Study in Human Lymphocytes In Vitro

ACCESSION No./MRID No.: (Not yet assigned)/na ["REF. 3" of Submission]

SPONSOR/TESTING LAB: Chevron/ICI Central Toxicology Lab

STUDY No./DATE: ICI Report No. CTL/P/1351, 9/3/85

TEST MATERIAL: Paraquat dichloride, 99.6% ai, a white solid, dissolved in saline for testing.

PROCEDURES:

While blood from 2 healthy donors (1 male, 1 female; both with low background of aberrations) was placed into suspension culture by established procedures, including the mitogen, PHA. Approximately 44 hours later, duplicate cultures were exposed to 10 concentrations of test material ranging from 0.75 to 3500 ug/ml, both in the absence and presence of a metabolic activation system (MA) consisting of the microsomal fraction (S9) from Aroclor 1254 treated male S-D rats, plus appropriate co-factors.

The clastogens, mitomycin-C (MC) and cyclophosphamide (CP) served as positive controls.

Two hours prior to harvest (72 hours from initiation), colchicine was added to all cultures, and microscope slide preparation made (4 per culture). Mitotic indices and chromosome damage (100 metaphases per culture) were recorded, and data analyzed by Fisher's Exact Test (with $p < 0.01$).

Results:

Severe cytotoxicity was observed in cultures exposed to the HDT (3500 ug/ml); dose-related decreases in mitotic indices were recorded at lower doses (2500, 1450, 1250, 350, and 125 ug/ml) [see tabulated summary attached].

Increases in simple chromosome damage (breaks and fragments) were found in non-activated cultures from both donors exposed to 1250 and as well as 2500 ug/ml, statistically significant only at the higher dose. However, one cell with multiple fragmentation and one with an interchange were observed at 1250 ug/ml, both from one donor. In the presence of S-9, statistically significant increases in damage were found at 1750 and 3500 ug/ml in cells from Donor 1, and at 2500 ug/ml in those from Donor 2. Whereas only simple breaks and fragments were recorded at less than the HDT, high-dose metaphase scoring included cells with multiple fragmentation and an interchange figure.

Positive control cultures (only 25 cells per culture examined) showed the expected increase in both simple and complex chromosomal damage.

Conclusions:

The authors concluded that:

"Paraquat dichloride does induce chromosome damage in the in vitro human lymphocyte cytogenetic assay, but only at dose levels where there is obvious cytotoxicity. No chromosomal damage was apparent at non-cytotoxic dose levels."

TB Evaluation:

ACCEPTABLE. Paraquat dichloride is a weak mutagen in human lymphocytes cultured in vitro (chromosome breaker) at cytotoxic concentrations.

PARAQUAT DICHLORIDE: CYTOGENETIC STUDY IN HUMAN LYMPHOCYTES IN VITRO *

	- S9			+ S9		
Donor	Dose (ug/ml)	%MI	%CA	Dose (ug/ml)	%MI	%CA
1 (Male)	0 (Saline) MC:0.5	9.5 6.0	1.0 40.0**	0 (Saline) CP:100	12.5 5.0	0.4 40.0**
	Para: 125	5.0	1.0	Para: 350	5.0	2.0
	1250	3.0	3.5	1750	4.0	5.0**
	2500	3.0	15.2**	3500	4.0	22.0**
	3500	1.5	(N/A)			
2 (Female)	0 (Saline) MC:0.5	11.0 8.0	1.0 60.0**	0 (Saline) CP:100	7.5 2.0	0.5 69.2**
	Para: 125	8.5	1.0	Para: 125	5.5	1.0
	1250	5.0	2.0	1250	6.0	1.0
	2500	5.0	10.5**	2500	3.0	8.5**
	3500	(Lethal)	(N/A)	3500	(Lethal)	(N/A)

LEGEND: - S9, cultures exposed in absence of metabolic activation
+ S9, cultures exposed in presence of metabolic activation
% MI, mean percent mitotic index
% CA, mean percent of abnormal cells (excluding gaps)

* Compiled from Tables 1 and 2 of the Report
** $p < 0.01$ (Fisher's Exact Test)

TOXICOLOGY BRANCH DATA REVIEW

Caswell: 634
EPA Chem. 061602

CHEMICAL: Paraquat Dichloride

STUDY TYPE: Mutagenicity - DNA Damage/Repair In Vitro (UDS/rat hepatocytes)

CITATION: Paraquat Dichloride: Assessment for the Induction of Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures

ACCESSION No./MRID No.: (Not yet assigned)/na ["REF. 4" of Submission]

SPONSOR/TESTING LAB: Chevron/ICI Central Toxicology Lab

STUDY No./DATE: ICI Report No. CTL/P/1339, 9/4/85

TEST MATERIAL: Paraquat dichloride, 99.6% ai, a white powder, dissolved in Williams incomplete medium "E" for testing.

PROCEDURES:

Rat hepatocytes from an untreated male rat (Alderly Park SPF albino) were allowed to attach to coverslips for 2 hours, then exposed for 19 hours to tritiated-thymidine (100 uCi) together with graded log concentrations of test material ranging from 10^{-2} to 10^{-9} M (3 coverslips per dose level). The coverslips were then washed, incubated overnight in culture medium containing unlabelled thymidine, and prepared for scoring silver grains (a measure of unscheduled DNA synthesis), according to referenced procedures. Diethylnitrosamine (DEN, 10^{-2}, 10^{-3}, 10^{-4} M) served as the positive control.

The assay was repeated once using hepatocytes from another animal.

Mean nuclear and cytoplasmic net grain values for the treated and test population for each experiment were then tested statistically for induction of UDS using one-sided analyses of variance against concurrent positive and negative controls.

Results:

Insufficient cells of normal morphology were provided at the higher concentrations of test material (10^{-3}, 10^{-2} M), reportedly due to cytotoxicity. At no dose in neither of the two experiments were mean net nuclear grain count values from test preparations (ranging from -1.06 to -7.17 in EXPT. 1, and from -4.02 to -8.12 in EXPT. 2) different from controls (-4.98 and -5.42, respectively), confirmed by analysis of variance. In contrast, DEN (10^{-3} M) induced the expected positive response (23.59 and 31.03 net nuclear grains).

Conclusion:

The authors concluded that paraquat dichloride was not genotoxic when tested for induction of UDS in primary rat hepatocyte cultures.

TB Evaluation:

ACCEPTABLE. Paraquat dichloride is negative in the HPC/UDS assay.

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TOXICOLOGY BRANCH DATA REVIEW

Caswell: 634
EPA Chem. 061602

CHEMICAL: Paraquat Dichloride

STUDY TYPE: Mutagenicity - Micronucleus Test in Mice

CITATION: An Evaluation of Paraquat Dichloride (Technical) in the Mouse Micronucleus Test

ACCESSION No./MRID No.: (Not yet assigned)/na ["REF. 5" of Submission]

SPONSOR/TESTING LAB: Chevron/ICI Central Toxicology Lab

STUDY No./DATE: ICI Report No. CTL/P/1369, 9/4/85

TEST MATERIALS: (I) Paraquat dichloride (analytical), 99.4% ai, an off-white solid, dissolved in dionized water for oral administration.

(II) Paraquat dichloride (technical), 33.07% paraquat ion, a brown liquid, dissolved in DW for oral administration.

PROCEDURES:

Juvenile (8-12 weeks) male and female C57BL/6 mice (5 per sex per treatment) were administered test materials once by oral gavage in two phases: Phase I, using both test materials, for determination of Median Lethal Dose; (MLD/7, as calculated on deaths over a 7-day period); Phase II, Micronucleus Test, using only test material II (Technical) at two doses, equivalent to 80% and 50% of the MDL/7. Cyclophosphamide (CP) served as the positive control for the micronucleus test.

Groups of Phase II animals were killed 24, 48 and 72 hours post-dose, and bone marrow cells processed for the determination of micronuclei in polychromatic erythrocytes (PCE), following standard (referenced) procedures. Results were analyzed statistically by Student's "t" test (one sided).

Results:

Phase I (MLD/7 Determination): Single doses of 51.75, 82.8 and 20.7 mg/kg of analytical grade paraquat dichloride (equivalent to 37.5, 50 and 150 mg/kg paraquat ion concentration) resulted in deaths at the high dose only (2/5 males and 5/5 females). The MLD for the paraquat ion (calculated graphically on the females, the more sensitive sex) was determined to be 105 mg/kg. No deaths occurred from acute oral dosing with the technical at 82.8 and 51.75 mg/kg paraquat ion concentration (stated to be equivalent to 80% and 50% of the MLD/7 levels, but actually 84 and 52.5 mg/kg, due to an acknowledged error in calculation).

Phase II (Micronucleus Test): No increase in mean micronucleated PCE (range 1.1 to 3.0/ 1000 per animal) over water controls (2.5, 2.9, and 2.1) at any sampling time was found at either dose of the technical. In contrast, CP-treated animals showed significantly increased incidences of micronuclei at all three sampling times ($p < 0.01$ at 24 and 48 hours; $p < 0.05$ at 72 hours).

The ratio of PCE to mature erythrocytes (a measure of cell cycle toxicity) was reduced from control values (32.7, 38.2, 34.4) by CP treatment at all sampling times, significantly ($p < 0.01$) at 48 and 72 hours. However, only small, non-significant reductions were observed in paraquat cultures, and only at the HDT.

Conclusions:

The authors concluded that "....paraquat dichloride (technical) is not clastogenic in the mouse micronucleus test."

TB Evaulation:

UNACCEPTABLE. Insufficient evidence is presented that the HDT affected erythropoiesis. The calculated MLD/7 (and nominally derived 80% and 50% there of) was based on an inadequate acute toxicity test.

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TOXICOLOGY BRANCH DATA REVIEW

Caswell: 634
EPA Chem. 061602

CHEMICAL: Paraquat Dichloride

STUDY TYPE: Mutagenicity - Sister Chromatid Exchange In Vitro (Chinese hamster DON Cells)

CITATION: Paraquat Dichloride: An In Vitro Sister Chromatid Exchange Study in Chinese Hamster Lung Fibroblasts

ACCESSION No./MRID No.: (Not yet assigned)/na ["REF. 6" of Submission]

SPONSOR/TESTING LAB: Chevron/ICI Central Toxicology Lab

STUDY No./DATE: ICI Report No. CTL/P/1392, 9/24/85

TEST MATERIAL: Paraquat dichloride, 99.4% ai, stated to be: "...a creamy grey /fawn powder," dissolved in physiological saline (0.85%) for testing.

PROCEDURES:

Duplicate monolayer cultures of DON cells (derived from Chinese hamster lung fibroblasts) were exposed for 3 hours to test material at 8 dose levels (concentration range, 1.2 to 2470 or 2450 ug/ml), both in the absence and presence of a metabolic activation system (MA) consisting of Aroclor 1254-stimulated microsomes (S-9) from male S-D rats plus appropriate co-factors. Cell cultures were then treated with brom deoxyuridine (300 ul), covered with aluminum foil and incubated for a further 20 hours. Two hours prior to harvest, colchicine was added, and the re-suspended cells processed for microscope slide scoring of sister chromatid exchanges (SCE), according to standard (referenced) procedures.

The ratio of first, second and third mitotic division cells (M_1: M_2: M_3) was calculated for each slide, to evaluate effects on cell cycle kinetics. Coded slides were then scored for SCE (both per cell as well as per chromosome), but only SCE/cell values analyzed by (one-sided) Student's "t" Tests.

Mitomycin-C (MC) and cyclophosphamide (CP) served as positive controls.

Results:

Doses above 124 ug/ml in non-activated cultures and greater than 245 ug/ml in S9-supplemented cultures were cytolethal (no mitotic cells). The mean mitotic indices (MI) in test cultures selected for analysis were comparable to saline controls (= 10.5) except at the HDT (124 ug/ml) in the absence of S9 (Report Table 1), where a value 24% of background was recorded; in the presence of S9, mean MI was reduced in all test cultures roughly equally (10.5 to 16.0) compared to control (= 17.0), except for a 56% reduction (to 7.5) at the intermediate dose of 24.5 ug/ml (Report Table 2). In addition, severe chromosomal damage (interchanges, fragmentation) was evident in S9 test cultures treated at the highest dose (245 ug/ml).

Statistically significant ($p < 0.01$) dose-related increases over the saline control SCE value (= 8.45 SCE/cell) were recorded in all non-activated cultures treated with paraquat, ranging from 11.51 SCE/ cell at the lowest dose (1.2 ug/ml) to 22.56 at the highest (124 ug/ml). Although not statistically analyzed, dose-related increases in SCE/chromosome (representing the severity of damage) were also observed (Report Table 5). Comparable though less extensive increases were recorded in S-9 supplemented test cultures; highly significant values ($p < 0.01$) of 9.01, 11.93 and 18.84 SCE/cell (compared to 7.62 in control) being recorded at only the three higher doses, 24.5, 123 and 245 ug/ml (respectively). The 8.33 and 8.55 values were also significant at the 5% level in cultures exposed to the lowest (1.2 ug/ml) and an intermediate (12.3 ug/ml) doses, but not (= 7.48 SCE/cell) at 2.5 ug/ml.

These increases in SCE were found in the absence of any severe perturbations in the cell cycle (defined by the ratios of M_1: M_2: M_3 cells, as a reduction not in excess of 20% $M_2/M_1 + M_3$). Both positive controls responded in the expected fashion, MC treatment resulted in 41.82 SCE/cell and CP treatment in 77.72 SCE/cell, both treatment showing severe damage as recorded on a per chromosome basis.

Conclusion

The authors concluded that the test material induced increased SCE in this assay, both in the absence and presence of metabolic activation, but more marked in the absence of S9.

TB Evaluation:

ACCEPTABLE. Paraquat dichloride induced increased SCE in Chinese hamster lung fibroblasts (DON cells) in vitro.