Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

008504

DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 412562-04
ID No.: 91 TX 0022
RD Record No.: S-396696
Caswell No.: 753
Project No.: 1-1718

Study Type: Mutagenicity - DNA Damage in Bacteria (Differential Toxicity in E. coli)

Chemical: Sodium Chlorate

Sponsor: Kerr McGee Chemical (for the SCTF)

Testing Facility: Life Science Research
Suffolk, UK

Title of Report: Sodium Chlorate: Assessment of Its Ability to Cause Lethal DNA Damage in Strains of Escherichia Coli

Authors: K. May

Study Number: LSR Schedule No.: SKR/004
LSR Report No.: 89/SKR004/0341

Date of Issue: September 5, 1989

TB Conclusions:

Positive for primary DNA damage, as demonstrated by differential toxicity in repair-deficient derivative strains of E. coli, as compared to the proficient parental strain, and without the requirement for metabolic activation (i.e., ±S9), at doses above 1000 ug/mL.

Classification (Core-Grade): ACCEPTABLE.

II. DETAILED REVIEW

A. Test Material - Sodium chlorate

Description: White crystals
Batch (Lot): (Not stated)
Purity (%): 99.9 (chromium content, 1.9 ppm)
Solvent/Carrier/Diluent: Distilled water (DW)

B. Test Organism - Bacterial cultures

Species: Escherichia coli (WP2) (tryp$^-$)
Strains: WP67 (uvrA$^-$/polA$^-$); CM871 (uvrA$^-$/recA$^-$/lexA$^-$)

C. Study Design (Protocol) - This study was designed to assess the genotoxic (differential toxicity) potential of sodium chlorate when administered in vitro (by spot test) to cultures of normal and repair deficient strains of E. coli, according to recognized (published) procedures, referenced in this Final Report.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were both provided.

D. Procedures/Methods of Analysis - Overnight (duplicate) broth cultures of the three E. coli strains were exposed to five concentrations of test article for 24 hr, and differential toxicity assessed by counting colonies per 20 ul spot after incubation both in the absence and presence of a metabolic activation system consisting of the post-mitochondrial (S9) fraction of liver homogenates from young CD male rats pre-treated with Aroclor 1254, plus NADP (H)-generating co-factors (S9-Mix).

In addition to negative controls (the antibiotic, Ampicillin, at 25 ug/mL, which is toxic to all strains by a non-genetic mechanism), the mutagens mitomycin C (MC, 0.05 ug/mL) and 2-aminoanthracene (AAnth, 5 ug/mL) were included as positive controls for, respectively, the nonactivated (-S9) and activated (+S9) test series.

Colony counts in test cultures were compared to relevant untreated (DW) control cultures to calculate coefficients of survival, C_s, by the formula:

$$C_s = \frac{\text{Mean \% survival of treated repair-deficient cells}}{\text{Mean \% survival of treated repair-proficient cells}}$$

E. Results:

Preliminary toxicity testing had demonstrated the test article reduced cell survival by 38% and 52% of solvent (DW) controls when the normal strain (WP2) was exposed for 2 hr to concentrations of 10,000 and 3160 ug/mL, respectively, but only in the presence of S9 (Report Appendix 1). After 18 hr, relative cell survival was reduced to 38%, 47% and 61% at 10,000, 3160 and 1000 ug/mL/+S9, or 15%, 29% and 34% at the same concentrations/-S9. Hence, 10,000 for 2 hr was selected as the HDT conditions for the main assay.

In the main assay, sodium chlorate proved more toxic to both repair-deficient strains (WP67 and CM871) than the proficient WP2, generating C_8 values less than 0.3, at dose levels of 1000, 3160 and 10,000 ug/mL, whether activated or not (Report Tables 1, 2, 3; Appendices 3, 4). Both direct (MC) and indirect (AAnth) mutagens produced significantly low C_8 values, confirming previous experiments performed in this lab.

The negative control (AMP) had roughly the same toxicity to all strains.

Hence, the study author concluded that sodium chlorate was positive in causing primary DNA damage without the requirement for activation, since it selectively increased toxicity (C_8 values < 0.3) in repair-deficient strains.

F. TB Evaluation: Acceptable

Attachment (Data Tables)

RIN 2906-01

DER/MRID No. 412563-04

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07/26/91
8/5/91

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DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 412562-05
ID No.: 91 TX 0022
RD Record No.: S-396696
Caswell No.: 753
Project No.: 1-1718

Study Type: Mutagenicity - DNA Damage/Repair in Mammalian Cells in vitro (HeLa/UDS)

Chemical: Sodium chlorate

Sponsor: Kerr McGee Chemical (for the SCTF)

Testing Facility: Roma Toxicology Center

Title of Report: Unsheduled DNA Synthesis (UDS) in HeLa S3 Cells in vitro

Authors: A.H. Seeberg

Study Number: 102002-M-02289

Date of Issue: September 27, 1989

TB Conclusions:

Negative for unscheduled DNA synthesis (UDS, as measured by liquid scintillation incorporation of tritiated thymidine in DNA) in human cells (HeLa-S3) exposed up to 10,000 ug/mL, with/without activation.

Classification (Core-Grade): ACCEPTABLE.

II. DETAILED REVIEW

A. Test Material - Sodium chlorate

Description: White crystals
Batch (Lot): (Not given)
Purity (%): 99.9 (chromium content, 1.9 ppm)
Solvent/Carrier/Diluent: Distilled water (DW)

B. Test Organism - Mammalian cell line

Species: Human, derived initially from uterine carcinoma
Strains: HeLa-S3
Source: Flow Lab. Ltd. (UK).

C. Study Design (Protocol) - This study was designed to assess the genotoxic (DNA damage/repair) potential of sodium chlorate for induction of unscheduled DNA synthesis (UDS) when administered in vitro to cultured human cells, according to a included protocol (Report/Appendix-I) consistent with the Agency Testing Guideline for such an assay.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were both provided.

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing to select appropriate doses, triplicate cultures of HeLa-S3 cells (in an arginine-free culture medium, supplemented with 10 mM hydroxyurea, in order to limit replicative, i.e., scheduled DNA synthesis) were exposed for 3 hr to graded concentrations of test article, together with tritiated thymidine (^{3}HTdR, 5u Ci/mL). Assays were conducted both in the absence and presence of a mammalian metabolic activation system consisting of the S9 fraction of homogenized livers from male S-D rats pre-treated with a mixed cocktail of enzyme inducers (phenobarbitone plus beta naphthoflanone). In addition to solvent (DW) controls, other cultures were treated with the mutagens, 4-nitro-quinoline oxide (NQO, 5 ug/mL) or benzo(a)pyrene (BaP, 2.5 ug/mL) to serve as positive controls for the non-activation (-S9) and activation (+S9) test series, respectively.

At the end of the treatment period, cells were pelleted, the DNA extracted by trichloracetic acid, and the uptake of TdR (a measure of unscheduled DNA synthesis) determined by liquid scintillation counting (Packard 4000), providing disintegration-per-minute (dpm) automatically (against an external standard and a previously constructed quench

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curve). The amount of DNA per tube was estimated by Burton colorimetry, and absolute values for DNA concentration determined by regression equations obtained from plots of concurrent standards.

Individual tube/treatment data were presented as dpm per microgram DNA, and group means (and their deviations) first normalized by square root transformation, then statistically analyzed by ANOVA.

The entire assay was repeated once.

The lab considers a test substance to be positive (inducing UDS) if it increases ^{3}HTdR incorporation to 50% (or more) greater than background controls at two consecutive dose levels, or at the highest practicable dose level. This increase should be confirmed in a repeat experiment.

E. Results:

Sodium chlorate was not toxic up to limit dosing at 10,000 ug/mL, (Report Tables 1, 2), which was the HDT selected for the main assay.

UDS data from all replicates were presented individually for each of the two main assays (Report Tables 3 to 6). In neither trial, however, did the test article produce any significant increase in UDS over concurrent (or historical) control (Report Table 7, attached to this DER). By contrast, both mutagens responded appropriately.

The study author concluded that sodium chlorate did not induce UDS in HeLa-S3 cells under the conditions of this assay.

F. TB Evaluation: Acceptable

Attachment (Data Tables)

ATTACHMENT

(Summary Data Tables)

SCHEDULE NO.: 102-002

Table 7

UNSCHEDULED DNA SYNTHESIS IN HeLa S3 CELLS

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Test Substance: Sodium Chlorate

Summary table

Mean incorporation of tritiated thymidine (DPM/ug DNA)

Nominal Dose-level (ug/ml)	Absence of S9		Presence of S9	
	Expt 1	Expt 2 *	Expt 1	Expt 2 *
0.00	89.2	120.7	122.9	199.8
100	84.2	110.1	127.5	191.4
316	78.7	100.2	122.1	205.6
1000	63.0	99.1	102.1	199.8
3160	57.3	85.1	106.3	186.3
10000	91.8	72.1	90.2	173.5

* Actual exposure concentrations may have been approximately 75% of the nominal dose as indicated by analysis of the stock solution. (See text, section 4.4)

Positive controls

4-NQO	1603.7	2559.8	NT	NT
B(a)P	NT	NT	215.5	386.4

Mean historical values for solvent control cultures in this laboratory based on the ten preceding studies (Mean ± S.D.):

98.36 ± 30.5 142.4 ± 31.2

NT : Not tested
4-NQO : 4-Nitroquinoline-N-oxide 5.00 ug/ml
B(a)P : Benzo(a)pyrene 2.50 ug/ml
The values given for the zero dose-level were obtained after treatment with the vehicle only.