1-Bromopropane (N-propyl bromide) is Not Mutagenic in an Ames Assay Using Closed Test Conditions & is Clastogenic in an *in vitro* Chromosome Aberration Assay

Carr J. Smith[1], Valentine O. Wagner, III[2], Shambhu Roy[2], Philip Atkins[2], Joseph H. Miller[1], Steven P. Anderson[1], Indu Kheterpal[1]

[1] Albemarle Corporation, Baton Rouge, LA | [2] BioReliance Corporation, Rockville, MD

Why were these assays conducted?

- Positive tumor results were reported in the two-year 1-bromopropane inhalation study conducted on B6C3F1 mice and F344/N rats by the US National Toxicology Program (NTP) in 2013.
- NTP puts great weight on the Ames test in evaluating mode of action (MOA) of chemicals.

Tumorigenicity of 1-BP in NTP Two-Year Inhalation Study (2013)

- Four categories of animals were exposed: male mice; female mice; male rats; and female rats. Dose-related skin neoplasms were observed in male rats only. Neoplasms of the large intestine were seen in both male and female rats, but in neither male nor female mice. Lung neoplasms were found in female mice only. Although dose-related neoplasms of the skin, large intestine and lung were reported, the incidence varied by sex and species.

Species/Sex	Skin Neoplasms	Large Intestine Neoplasms	Lung Neoplasms
Male Mice	Negative	Negative	Negative
Female Mice	Negative	Negative	Positive
Male Rats	Positive	Positive	Negative
Female Rats	Negative	Positive	Negative

Table 2: Results from NTP 2-Year Inhalation Study on 1-Bromopropane

NTP Emphasis on Ames Test Results

"DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone..."

- National Toxicology Program. US Department of Health and Human Services. Report on carcinogens, monograph on 1-bromopropane. September 2013.

Final Ames Assay and *In Vitro* Chromosome Aberration Assay GLP Reports

- The following link is directed to Albemarle's public comments to USEPA regarding the draft risk assessment of 1-bromopropane: https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2015-0084-0021
- Please see Appendix III for final BioReliance Ames test report; Appendix IV for final BioReliance Analytical Protocol report; and Appendix V for final BioReliance *in vitro* chromosome aberration report.

Abstract

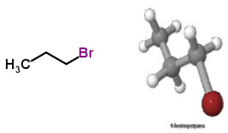
1-Bromopropane (1-BP) (CASRN 106-94-5) is an important industrial chemical. The National Toxicology Program (NTP) has classified 1-BP as "reasonably anticipated to be a human carcinogen." NTP has stated that 1-BP is a direct-acting mutagen in *Salmonella* strains TA100 and TA1535. In the current study, a closed preincubation system was employed to test 1-BP for mutagenicity in *Salmonella* strains TA98, TA100, TA1535 and TA1537, and in *Escherichia coli* WP2 *uvrA* in both the presence and absence of metabolic activation. Chemical analysis of the assay tubes was conducted to ensure that the bacterial cells were exposed to the 1-BP as intended. Although 1-BP was toxic to the bacteria beginning at 3000 or at 5000 µg per plate, it did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9. In contrast, under similar closed assay conditions 1-BP was clastogenic in the *in vitro* chromosome aberration assay conducted using human peripheral blood lymphocytes (HPBL). The *in vitro* mammalian chromosome aberration assay was conducted using standard procedures as per OECD testing guideline 473. At this time, the mechanism of action underlying the reported tumorigenicity of 1-BP in rodents remains unclear.

Keywords: 1-bromopropane, Ames test, Chromosome Aberration, Analytical verification

Summary of *In Vitro* Chromosome Aberration Assay Results

- Due to time and length constraints, this poster will focus on the Ames results, with the chromosome aberration assay results to be described later.
- Under closed assay conditions 1-BP was clastogenic in the *in vitro* chromosome aberration assay conducted using human peripheral blood lymphocytes (HPBL). The *in vitro* mammalian chromosome aberration assay was conducted using standard procedures as per OECD testing guideline 473.

Structure of 1-Bromopropane (CAS# -94-5)



1-Bromopropane (1-BP) is Volatile (Necessitates Closed System Assays)

- Vapor pressure 19.5 kPa (at 20 degrees Centigrade).
- In contrast, vapor pressure of 2-bromopropane is 32 kPa (at 20 degrees Centigrade).
- While 1-bromopropane is volatile, the synthetic impurity 2-bromopropane is more volatile.

Purity and Source of 1-BP (Test Article)

- The 1-bromopropane sample provided by Albemarle Corporation to BioReliance Corporation was produced in a dedicated process in Albemarle's manufacturing plant located in Magnolia, AR.
- The sample analyzed at 99.99 wt% purity by gas chromatography (GC).
- The principle impurities were isopropyl bromide (65 ppm) and water (27 ppm).

Test System

- The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames *et al.* (1975) and *Escherichia coli* WP2 uvrA as described by Green and Muriel (1976).
- Aroclor 1254-induced rat liver S9 was used as the metabolic activation system.

Plating and Scoring Procedures

- The test system was exposed to the test article via the preincubation methodology described by Yahagi *et al.* (1977). One half (0.5) milliliter of S9 or sham mix, 100 µL of tester strain (cells seeded) and 25 µL of vehicle or test article dilution in ethanol were added to 13 X 100 mm glass culture tubes pre-heated to 37±2°C. When plating the positive controls, the test article aliquot was replaced by a 50 µL aliquot of appropriate positive control as listed in Tables 4 and 5. Tubes receiving test article were capped (screw caps) during the preincubation period.

Results from Initial Toxicity-Mutation Assay

- In the initial toxicity-mutation assay, the maximum dose tested was 5000 µg per plate; this dose was achieved using a concentration of 200 mg/mL and a 25 µL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate.
- No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. No precipitate was observed.
- Cytotoxicity was observed at 5000 µg per plate with all tester strains. A dose level is considered toxic if it causes a >50% reduction in the mean number of revertants per plate relative to the mean vehicle control value (this reduction must be accompanied by an abrupt dose dependent drop in the revertant count) or a reduction in the background lawn (code 3, 4, or 5). Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg per plate.

Confirmatory Mutagenicity Assay

- In the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 50, 150, 500, 1500, 2000, 3000 and 5000 µg per plate. No precipitate was observed. Toxicity was observed at 5000 µg per plate with all tester strains. However, due to technical errors, the entire assay was repeated.

Retest of Confirmatory Mutagenicity Assay

- In the retest of the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 50, 150, 500, 1500, 2000, 3000 and 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 3000 or at 5000 µg per plate with all tester strains.
- Under the conditions of this study, test article 1-bromopropane was concluded to be negative in the Bacterial Reverse Mutation Assay.

Testing Up to Toxicity in First Ames Assay

- Despite the low recovery of test article in the treatment tubes, toxicity was observed in the assay. In the initial trial, toxicity was observed at 5000 µg per plate with all tester strains. Since this trial also served as a dose range-finder trial, the doses were spaced at half-log intervals (5000, 1500, 500, 150, 50, 15, 5.0 and 1.5 µg per plate).

Testing Up to Toxicity in Repeat Ames Assay

- For the second trial, the doses were spaced more closely (5000, 3000, 2000, 1500, 500, 150 and 50 µg per plate), and toxicity was observed beginning at 3000 or at 5000 µg per plate with all tester strains. In all trials, there were no indications of mutagenic activity.

Analysis of 1-BP in Dosing Formulations and Treatment Samples

Analyzed and quantified 1-BP by a validated GC-FID method to:

- Assess accuracy of the dosing formulations in ethanol and in the treatment samples.
- Ensure exposure of bacterial samples to 1-BP.

Analysis of 1-BP in Dosing Formulations

Trial No.	Concentration of 1-BP in Ethanol Control (mg/mL)	Nominal Concentration of 1-BP in Dosing Samples (mg/mL)	% of Nominal Concentration Measured
1	ND	0.06	104
1		200	96.8
2	ND	2	102.1
2		200	103.1
3	0.03	2	96.9
3		200	97.9

- All formulation samples were prepared in ethanol.
- Dosing formulation samples were found to be 97-104% of the target indicating that Ames test system was accurately dosed.
- Dosing formulation samples were also found to be stable for >3 h, covering the period of use.

Analysis of 1-BP in Treatment Samples

Trial No.	Concentration of 1-BP in Vehicle Control	Nominal Concentration of 1-BP in Treatment Samples (mg/mL)	% of Nominal Concentration of 1-BP Measured at T=0 min	% of Nominal Concentration of 1-BP Measured at T=90 min	% Concentration of 1-BP Measured at T=90 min relative to T=0
1	ND	0.95	10.7	12.0	111.9
1		9.5	8.5	10.0	117.4
2	ND	0.95	37.2	2.6	6.9
2		9.5	9.2	2.1	22.8
3	ND	0.95	7.5	4.6	61.1
3		9.5	4.1	2.9	69.9

- All treatment samples were prepared in Sham mix (100 mM phosphate buffer, pH 7.4) and diluted with ethanol to a final concentration of 10% Sham mix by volume.

Analysis of 1-BP in Treatment Samples

- Detectability of 1-BP in Sham mix is low (8 ± 2.5 % at T=0 min).
- Considerable fluctuation in 1-BP measured at the beginning (T=0 min) and at the end of the dosing period (T=90 min).
- This is due to the test article's limited aqueous solubility, volatility and the interaction of 1-BP with the test system.
- Toxicity observed at nominal 1-BP concentration of 4.8 and 8 mg/mL in the Ames Assay.
- 1-BP induced structural chromosome aberrations starting at a nominal concentration of 0.6 mg/mL.

Conclusion

The mutagenicity result is valid because the test system was exposed up to toxic doses as required by OECD 471 and the recommendations of Gatehouse *et al.* (1994), and Mortelmans and Zeiger (2000).