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# EBPI Analytics Report:

**Date:** December 15, 2012 **Quote ID:** 12-2455
**Client:** A + Chemical Dev **Project #:** 121512

**Details of Analysis Performed:**

The client, A + J Chemical, submitted two (2) samples and one (1) Lab Blank to EBPI for mutagenicity analysis. The identity and characteristics of the samples were unknown and were labelled by the client prior to arrival. The samples were delivered to EBPI Analytics via ground mail shipment for mutagenicity testing using the Ames test MOD ISO™ fluctuation method. The package was received on December 10, 2012 and processed according the EBPI Analytics chain of custody protocols **(see attached COC forms)**. No refrigeration method was found in the shipment and it was noted that the sample seals were received intact. Aluminum foil was used as a primary cap to seal the viles and EBPI determined that the probability of contact between the sample and the plastic cap was extremely low. Each sample was packaged separately in an amber glass container. Sample volumes were estimated at 1 mL although some variations between samples were noted upon arrival. In particular, the volume of the Lab Blank sample was noted to be less than the other vials. The samples were tagged with care upon receipt. Sample temperature was measured upon receipt. Sample temperatures upon receipt were noted to be warmer than desired. Each solid sample was dissolved into sterile DMSO prior to analysis at the maximum allowable testing concentration of 5 mg/mL (OECD 471). Final sample concentrations were neutralized to pH 7.0 using sterile HCl. The samples were logged in to EBPI Analytics storage according to COC procedures. Each sample was renamed accordingly: **Unknown Sample (14) = Sample 1; Sunknown Sample (114) = Sample 2.** All samples were placed immediately into sample fridge for storage at 4 °C.

**Each sample** was tested using the Ames test MOD ISO fluctuation method with *Salmonella typhimurium* bacteria, strain TA98. Homogenized rat liver S9 fraction (lot number# 121543) with necessary cofactors (EBPI 2012) were included for bioactivation as per client request according to OECD 471 guidelines. The test strain and specific experimental setup were specified by the client prior to starting the assays and verified by verbal and written communication. Samples were run according to standard operating procedures (EBPI 2011) using the MOD ISO method including S9 bioactivation. Each sample was tested using the MOD ISO procedure, both in the presence and absence of S9 bioactivation, in duplicate by adding 200, 100, 50 and 25 µL of sample per exposure well (total volume 2000 µL). When S9 bioactivation was employed, the total volume of the exposure wells were slightly larger (2068 µL). As the analyte concentration within each sample was not known, dosages were selected based on consistency with previous experiments at EBPI Analytics and confirmed by the client prior to the start of testing. Negative controls were run for each test using the maximum concentration of DMSO and the Lab blank samples (100 µL) to establish independent baseline activity rates. The **Lab blank** sample submitted by the client was also run separately to check sterility and add an extra standard to the assays. Positive controls and

Laboratory results from Environmental Bio-Detection Products Inc. are for research purposes only and are not to be used for medical advice, diagnosis, or treatment. Test samples are screened as stated above through internationally recommended methods based on samples as provided by the client and received at our facility. EBPI will hold samples after delivery to our facility at 4 $^0$C until analysed unless otherwise instructed. EBPI is not responsible for changes in matrixes or test samples during transport or storage prior to analysis. Test samples are analysed based on client's specified dose range and method of analysis

**Conclusions and Future Work:**

It appears from this analysis that only **Sample 2 was weakly mutagenic to TA98 bacteria in the presence of S9 bioactivation**. The other positive responses observed did not exceed the necessary acceptance criteria with only one replicate per dose level. **Sample 1** produced some increase in reversions over background levels in the absence of S9 but was classified as possibly mutagenic when the variation of negative controls was factored in. Importantly, the lab blanks did not produce any significant mutagenic response or cytotoxicity at the concentrations tested which helped to validate the responses observed in the samples.

EBPI Analytics recommends the following actions for future studies:

1) Increase the amount of dilutions tested to produce a better dose response relationship and validate mutagenic responses observed, acquire coefficients of variation and improve the MOD-ISO fluctuation assay for the samples tested.
2) Increase the dose levels to observe potential responses at higher concentrations
3) Perform statistical analysis on sample replicates to substantiate mutagenic responses observed during testing.

Thank you very much for your continued work with EBPI Analytics. If you have any questions regarding the contents of the report, please contact the project lead at awitham@biotoxicity.com or 1-905-826-8378.

Sincerely,

Dr. Aaron Witham
Lead Toxicologist, EBPI

**References:**

1) **OECD 471** OECD Guidelines for Test of Chemicals (Bacterial Reverse Mutation Test)
2) **EBPI SOP MOD ISO Fluctuation method (2011)**
3) **EBPI acceptance criteria Mutagenicity Assays (2011)**

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**Date:** 12/5/2012

**Bioassay:** Ames test, fluctuation method MOD ISO
TA98 w/wout S9

**Table 1**

| **Ames Test** | | | **Project Title**: A + J Chemical | | | |
|---|---|---|---|---|---|---|
| **Controls** | | | **Strain validation criteria** | | | |
| **Date:** | | 10-Dec-12 | | | | |
| **Tech:** | | Bhavna Radia | | | | |
| **Quality Control** | | | **Revertants (plate)** | | | |
| **Strain** | **Control** | **S9** | **Day 3** | **SD** | **Criteria** | **Acceptance** |
| **TA98** | BL | – | 0 | 0 | 0 revertants | Y |
| **TA98** | NC | – | 2 | 1 | ≤ 12 revertants | Y |
| **TA98** | PC | – | 47 | 0 | ≥ 4X BC | Y |
| **TA98** | BL | + | 0 | 0 | 0 revertants | Y |
| **TA98** | NC | + | 4 | 0.25 | ≤ 12 revertants | Y |
| **TA98** | PC | + | 48 | 0 | ≥ 4X BC | Y |

| **Legend** | | |
|---|---|---|
| | **NC (negative control)** | Wells contain test vehicle or water and bacteria. No sample |
| | **PC (positive control)** | Wells contain a calibrated amount of known mutagen and bacteria. |
| | **BL (solvent control)** | Wells contain test vehicle and no bacteria. |

| **Quality Control Checks** | **Y** | **N** |
|---|---|---|
| **1. Solvent or matrix control demonstrates sterility** | **X** | |
| **2. Difference between Negative Controls does not exceed 5%** | **X** | |
| **3. Coefficient of Variation (CV) of triplicate testing at EBPI Analytics does not exceed 30%** | | |
| **4. Results are validated to a 95% confidence interval** | **X** | |
| **5. Positive Control Performance ( > 25 revertants Day 3/ 4x BG)** | **X** | |

## Table 2

**Ames Test**

**Project Title**: A + J Chemical December 2012

**TA98 ± S9 Addition**

**Date:** 10-Dec-12

**Tech:** Bhavna Radia

| Sample ID | [C] or Dilution (µL/2000 µL total exp) | Strain | S9 | Revertants (Standardized to NC) Day 3 | CV Day 3( %) | Significance |
|---|---|---|---|---|---|---|
| NC | 0 | TA98 | – | N/A | 0 | |
| PC | 50 | TA98 | – | 24.00 | 0 | Y |
| Sample 1 | 200 | TA98 | – | 3.25 | 0 | Y |
| Sample 1 | 100 | TA98 | – | 2.00 | 0 | Y |
| Sample 1 | 50 | TA98 | – | 2.00 | 0 | Y |
| Sample 1 | 10 | TA98 | – | 1.00 | 0 | |
| Sample 2 | 200 | TA98 | – | 1.25 | 0 | |
| Sample 2 | 100 | TA98 | – | 1.50 | 0 | |
| Sample 2 | 50 | TA98 | – | 1.00 | 0 | |
| Sample 2 | 10 | TA98 | – | 1.00 | 0 | |
| NC | 0 | TA98 | + | N/A | 0 | |
| PC | 50 | TA98 | + | 10.00 | 0 | Y |
| Sample 1 | 200 | TA98 | + | 1.25 | 0 | |
| Sample 1 | 100 | TA98 | + | 2.00 | 0 | Y |
| Sample 1 | 50 | TA98 | + | 1.25 | 0 | |
| Sample 1 | 10 | TA98 | + | 1.00 | 0 | |
| Sample 2 | 200 | TA98 | + | 3.75 | 0 | Y |
| Sample 2 | 100 | TA98 | + | 3.25 | 0 | Y |
| Sample 2 | 50 | TA98 | + | 2.75 | 0 | Y |
| Sample 2 | 10 | TA98 | + | 1.00 | 0 | |
| Lab Blank | 200 | TA98 | | 1.25 | 0 | |
| Lab Blank | 100 | TA98 | | 1.50 | 0 | |
| Lab Blank | 200 | TA98 | + | 1.00 | 0 | |
| Lab Blank | 100 | TA98 | + | 0.75 | 0 | |

**Figure 1: Results for Sample 1 and Sample 2 submitted by A + J Chemical, December 2012. Samples were run using the MOD ISO Fluctuation method for the Ames test with TA98 bacteria ± S9 bioactivation added.**