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REPORT ON

NAPHTHENIC ACIDS: UPDATE ON MAMMALIAN TOXICITY OF SUNCOR CT WATER

Submitted to:

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EXECUTIVE SUMMARY

This memorandum provides an update on recent research conducted by Suncor Energy to assess the genotoxic potential of naphthenic acids.

In all, three studies were conducted (two *in vitro* and one *in vivo*). The SOS Chromostest, an *in vitro* bacterial assay, indicated a lack of genotoxicity in fresh and aged CT water. In contrast, the Ames test, also an *in vitro* bacterial assay, resulted in elevated frequencies of revertant colonies for both fresh and aged CT water (but more so in aged CT water) suggestive of mutagenic activity. As a result of this equivocal *in vitro* screening data, the mouse micronucleus assay was employed to further examine genotoxicity. This assay used *in vivo* exposure in the intact mammalian system, a more relevant and applicable system to evaluate genotoxic risk to wildlife and humans. The results of this latter test clearly indicated a lack of genotoxicity.

Collectively, the results of these studies provide a strong weight of evidence indicating *whole CT water, of which the dominant organic chemical form is naphthenic acids, is not genotoxic in nature.* Because whole CT water was tested, these results also indicate that *the mixture of naphthenic acids together with other less prevalent constituents of CT water is also not genotoxic.*

This new information reduces the overall uncertainty in predicting environmental health risk from naphthenic acids released via oil sands operations. It indicates that CT water and/or naphthenic acids are not toxic to genetic material and therefore unlikely to cause carcinogenic effects or birth deformities (teratogenic effects), and that any other potential forms of toxicity likely require a threshold dose in order to be manifested.

The present findings provide important information (i.e., lack of genotoxicity in CT water and discharged naphthenic acids) that further argues the potential direct and indirect impacts from CT water releases are indeed low and likely negligible.

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1 BACKGROUND

Naphthenic acids are a complex mixture of water soluble organic substances present in petroleum, oil sands bitumen and crude oils. These substances are a major constituent of oil sands industry process water in northeastern Alberta. They are the dominant organic compounds in water released from consolidated tailings (CT) water at Suncor's oil sands facility. Chemical analyses indicate there are hundreds of naphthenic acids that vary in molecular weight, structure and concentration in CT water. The *total* concentration of naphthenic acids in Suncor's CT water is in the range of 60 to <100 mg/L (CEATAG 1998), with most recent chemical analysis results indicating concentrations of 78 and 62 mg/L, respectively (ETL 1997; 1998).

Existing toxicological data on naphthenic acids indicate these compounds have a relatively low toxicity in situations where the exposure is short (i.e., acute exposure). However, previous evaluations of the mammalian toxicity of these compounds, conducted for purposes of environmental impact assessments, have indicated that the database is incomplete with respect to potential genotoxic effects and effects from chronic low level exposure. This data gap contributed a degree of uncertainty when predicting human and wildlife health risks from environmental releases of CT water. While some have attempted to infer naphthenic acid toxicity from that reported for methylcyclohexane (CanTox 1993), the diversity of naphthenic acids structure is substantial and such inference may not be justified. There has been recognition by both Suncor and regulators of the need to assess the toxicity of these compounds in a way that is relevant to potential chronic low level environmental exposure.

To this end, Suncor undertook three studies to assess whether CT water, and its associated naphthenic acids content, is genotoxic. Addressing genotoxicity as the initial step in reducing the uncertainty of naphthenic acids mammalian toxicity was pursued because:

- It addresses whether these compounds have a threshold or non-threshold dose-response relationship (that is, it largely discriminates between chemicals which may theoretically exert a health risk by causing a self-propagating gene mutation via exposure to a single molecule versus a substance which requires a threshold level of exposure before an effect is manifested); and
- It provides good insight concerning the potential for genetic-based birth deformities (i.e., teratogenic effects).

2 SUMMARY OF RESULTS

The three studies conducted to address genotoxicity included:

- SOS Chromotest (*in vitro* exposure, bacterial test species);
- Ames Test (*Salmonella* Reverse Mutation Assay, *in vitro* exposure, bacterial test species); and
- Mouse Micronucleus Assay (*in vivo* exposure involving an intact whole mammalian system)

This battery of tests was employed because it provides a broader base of evidence for interpreting genotoxicity than can be offered by any one study alone and is consistent with testing strategies required by federal regulatory bodies for approval of new chemicals in Canadian commerce under the Canadian Environmental Protection Act (CEPA). The comprehensive results with raw data have been reported in two separate documents, the first addressing the *in vitro* studies (RSG 1998a) and the second addressing the *in vivo* study (RSG 1998b). The executive summary from each of these reports is provided in Appendix I.

2.1 SOS CHROMOTEST

The SOS-Chromotest is based on a genetically altered strain of *Escherichia coli* that produces and releases the enzyme β–galactosidase in response to mutagens. A doubling in β–galactosidase activity relative to controls is the basis for a positive test result in the SOS-Chromotest and is an indication of genotoxicity. The test has been optimized to be highly sensitive by disabling the DNA repair mechanism and enhancing the cell permeability. Both fresh and aged CT water were evaluated at 6 concentrations (1%, 3%, 9%, 30%, 100% and 200%) with and without a mammalian metabolic activation (i.e., S9). The results suggested that CT water (both fresh and aged) was not genotoxic, since β–galactosidase activity in test treatments was similar to controls (RSG 1998a).

2.2 AMES TEST

The Ames Test is based on the detection of elevated frequencies of bacterial colonies (in test treatments relative to controls) of four strains of *Salmonella*, which have previously been mutated to modify their nutritional requirements for certain essential compounds. Detection of elevated numbers of colonies from any of the test strains (compared to controls) infers the previously mutated test strain has undergone another mutation reverting back to its original nutritional status (i.e., "reverse" mutation). The test strains of *Salmonella* have been

optimized to be highly sensitive by enhancing cell permeability and disabling the DNA repair mechanism, thereby preventing a mutation caused by the test chemical from being repaired and going undetected. Additionally, the test employs a mammalian metabolic activation (S9) to address the potential creation of mutagenic mammalian metabolites, that otherwise may not be produced by these bacteria.

Similar to the study described for SOS Chromotest, the Ames test employed both fresh and aged CT water at 6 concentrations (1%, 3%, 9%, 30%, 100% and 200%). The results indicated that both fresh and aged CT water were genotoxic to the four strains of *Salmonella* tested with and without metabolic activation. However, there was no dose-response relationship, which is a critical factor in validating a clear cause-effect relationship. Additionally, a cytotoxic effect was observed at certain concentrations. The apparent mutagenic and cytotoxic potential was higher for the aged CT water than the fresh CT water (RSG 1998a).

2.3 MOUSE MICRONUCLEUS TEST

The third study focused on aged CT water because the Ames assay suggested aged CT water might yield a greater effect than fresh CT water.

Unlike the previous *in vitro* tests, the mouse micronucleus test employs *in vivo* exposure using mice as the mammalian system. This design confers greater relevance to humans and wildlife than the previous studies, and therefore confers the greatest weight of evidence to the overall results. The test principle involves dosing animals at various levels (in this case by a single oral dose of either 20, 2 or 1 g/kg), allowing various times of *in vivo* incubation (e.g., 24, 36 or 48 hours), extraction of bone marrow and analysis of the prevalence of micronuclei in polychromatic erythrocytes compared to controls treatments. Micronuclei are remnants of genetic material that has dislodged as a result of DNA damage (breaks) and become isolated during cell replication. Elevated levels of micronuclei in test treatments provide evidence that the test chemical is clastogenic (i.e., able to directly disrupt DNA), or affects the spindle apparatus that separates chromosomes during cell replication.

The micronucleus study found that none of the test treatments (i.e., doses of 20, 2 and 1 g/kg) resulted in elevated levels of micronuclei compared to controls. Positive control animals dosed with cyclophosphamide indicated that test mice were sensitive to substances known to cause micronuclei, thereby validating the test protocol (RSG 1998b).

3 RELATIONSHIP OF FINDINGS TO ENVIRONMENTAL RISK ASSESSMENT OF CT WATER RELEASE

Previous human and wildlife health risk assessments of constituents released in CT water were reported in Suncor's EIA submissions for Steepbank Mine and Project Millennium. In that report, human and wildlife health risks for metals and PAHs in CT water were found to be negligible. However, it was noted that there was uncertainty surrounding the significance of naphthenic acids to the overall assessment of direct and indirect health risk from water releases, for both wildlife and humans. The potential impacts were therefore categorized as "low" rather than "negligible" because:

- the uncertainty for genotoxic and chronic toxicity endpoints disallowed a conclusion of "negligible impacts" based on the criteria defined for the EIA; and
- the existing acute toxicity data coupled with field observations over the operating time frame of existing facilities suggested no adverse impacts on wildlife or human health in the oils sands region.

The present findings provide important information (i.e., lack of genotoxicity in CT water and discharged naphthenic acids) which suggests that potential direct and indirect impacts from CT water releases are indeed low and likely negligible.

GOLDER ASSOCIATES LTD.

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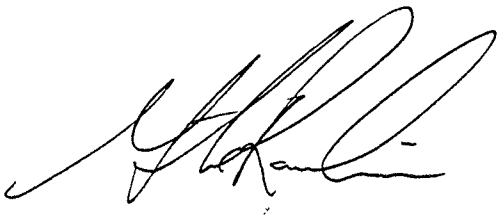

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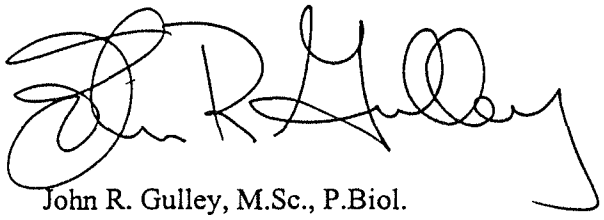

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APPENDIX I

EXECUTIVE SUMMARIES FOR MUTAGENICITY REPORT

AND

MOUSE MICRONUCLEUS TEST

EXECUTIVE SUMMARY

RSG CONSULTEST INC. was retained by **Golder Associates Ltd.** to provide mutagenicity testing of fresh and aged consolidated tailings water (CTW) produced from the Suncor Energy Inc. Oil Sands Operations in Fort McMurray, Alberta. The purpose of this testing was to provide mutagenicity data in support of Suncor's Environmental Impact Assessment of the Oil Sands Operation, under the Alberta Environmental Protection Act (AEPA).

Mutagenicity Testing

The following mutagenicity testing results for CTW were determined:

Reverse Mutation Assay – Fluctuation Procedure

- Fresh and aged CTW were tested in order to determine their potential to induce mutations in four histidine-dependent auxotrophs of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537), with and without metabolic activation (S9). Both fresh and aged CTW were evaluated at 6 concentrations (1%, 3%, 9%, 30%, 100% and 200%). Statistically significant differences in revertant frequencies were detected in TA98, TA100 and TA1537 tester strains at 200% and TA1535 at 1%, 3%, 9% and 200% exposed to fresh CTW with metabolic activation. No difference in revertant frequencies were observed in any of the tester strains exposed to fresh CTW without metabolic activation. However, cytotoxicity was observed in three of four tester strains (TA98, TA100 and TA1535) exposed to fresh CTW at 200%, without metabolic activation. Cytotoxicity was not observed in any of the strains with metabolic activiation, which may suggest that the cytotoxicity of the fresh CTW was reduced by the presence of the S9 mixture.

  Frequencies of revertants were also significantly higher than controls in TA98 and TA1535 strains at 30%, 100% and 200% and the TA1537 strain at 30% and 100% aged CTW with metabolic activation. Significant differences in revertant frequencies were also detected in the TA98 strain at 30% and 100%, the TA100 strain at 9%, 30% and 100% and the TA1535 strain at 9% and 30% aged CTW without metabolic activation. Cytotoxicity was observed in all tester strains exposed to the higher concentrations of aged CTW without metabolic

activation. Cytotoxicity was also observed in all strains with metabolic activation, though to a lesser degree than that observed without metabolic activation. The potential mutagenicity of both fresh and aged CTW was not dose related.

Based on these results, positive mutagenicity findings were observed in all four tester strains exposed to fresh CTW with metabolic activation, but mutagenicity was not observed in the absence of metabolic activation. Cytotoxicity was also observed in all tester strains exposed to fresh CTW at 200%, without metabolic activation. Positive mutagenicity findings were also observed in all four tester strains exposed to aged CTW with and without metabolic activation. These results suggest that both fresh and aged CTW are mutagenic. The mutagenic and cytotoxic potential was higher for the aged CTW than the fresh CTW.

SOS Chromotest

- Fresh and aged CTW were tested in order to determine their potential to induce mutations with and without metabolic activation, in a strain of *Escherichia coli* with its SOS promoter genetically altered to induce the synthesis of β-galactosidase. Both fresh and aged CTW were evaluated at 6 concentrations (1%, 3%, 9%, 30%, 100% and 200%). β-galactosidase activity remained comparable to solvent control in both fresh and aged CTW at all concentrations tested, with or without metabolic activation. Based on these results, no mutagenicity was detected with the SOS-Chromotest for either fresh or aged CTW, with or without metabolic activation.

Good Laboratory Practice Compliance

All laboratory work undertaken for this project was done using Good Laboratory Practice procedures.

Quality Assurance-Quality Control

Quality assurance and quality control measures were undertaken as part of Good Laboratory Practice compliance. Most quality assurance material is included in Sections 3, 4 and 5; however, any materials not included will be kept in the RSG CONSULTEST INC. archives for future reference.

EXECUTIVE SUMMARY

RSG CONSULTEST INC. was retained by **Golder Associates Ltd.** to provide a mouse micronucleus test of Suncor CT Water. The purpose of this testing was to provide data to assess the mutagenicity of the Suncor CT Water.

Mouse Micronucleus Test

The following results were determined:

- Five groups of mice were studied - high, intermediate and low test groups and negative and positive control groups. Each group consisted of 15 males and 15 females. The three test groups received the test article at 20.0, 2.0 and 1.0 g/kg respectively. The test article was administered orally by gavage. A dose of 20.0 g/kg is the maximum dose, by volume, that can be administered orally to a ~25 g mouse. The negative control group (WFI) was gavaged orally with the same volume of 20.0 g/kg as the test article. The positive control group received cyclophosphamide at 300 mg/kg orally. Ten animals from each group were sacrificed at 24, 36 and 48 hours after dosing. Bone marrow was recovered at each time point from both femora of all animals. Bone marrow smears were prepared, fixed and stained (May-Grunwald/Giemsa) for evaluation. 2000 polychromatic erythrocytes per animal were scored for the presence of micronuclei. In addition, a number of normochromatic erythrocytes with micronuclei per 2000 polychromatic erythrocytes were scored and the polychromatic-normochromatic ratio was established. The presence/absence of micronuclei was also confirmed in the positive control group at 24 hours, and the high test groups at 24, 36 and 48 hours by applying DNA specific stain (Fuelgen stain). The number of micronuclei were tabulated and analyzed for statistically significant difference (at $p=0.001$) using Kruskal-Wallis One Way Analysis of Variance on Ranks. There was a statistically significant increase in the number of micronuclei in the positive control group when compared to the test article and the negative control groups for all three time points. There was no statistically significant difference in the number of micronuclei between the test article and negative control groups for all three time points. Based on the above results, the

test article, Suncor CT Water, was found to be negative in the Mouse Micronucleus Test at 20.0, 2.0 and 1.0 g/kg, administered orally, at the 24, 36 and 48 hour time points.

Good Laboratory Practice Compliance

All laboratory work undertaken for this project was done using Good Laboratory Procedures.

Quality Assurance-Quality Control

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