# Assessment of DNA-Damaging Potential of *Acorus calamus* Rhizome Oil

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## Abstract

**Background:** *Acorus calamus* rhizome oil contains phenylpropanoid isomers (α-, β-, γ-asarone) that have been associated with genotoxic and carcinogenic effects in some experimental systems and have prompted regulatory restrictions. We performed a combined chemical and *in vitro* genotoxicity assessment of a hydro-distilled *A. calamus* rhizome oil (Kashmir origin) to inform a weight-of-evidence safety evaluation.

**Methods:** The test oil was characterized by HPLC and LC–MS/MS for α-, β- and γ-asarone content. An *in vitro* genotoxicity battery was applied following OECD guidance: the bacterial reverse mutation (Ames) test (TA98, TA100, TA102, TA1535, TA1537; ±S9), the cytokinesis-block *in vitro* micronucleus test (IVMNT) in human peripheral blood lymphocytes (short ±S9 and extended -S9) and, the L5178Y TK+/− mouse lymphoma assay (MLA; short- and long-term exposures ±S9). Cytotoxicity, precipitation, pH and osmolality were recorded and acceptance criteria/historical control ranges were met.

**Results:** Chemical analysis showed the sample to be β-asarone dominant (40.75%) with lower α-asarone (4.16%); γ-asarone was below the detection limit. In the Ames assay, no biologically or statistically reproducible increase in revertant colonies was observed in any strain up to the highest non-cytotoxic concentrations (no mutagenicity; cytotoxicity apparent ≥1,500 µg/plate). The IVMNT revealed no statistically significant or biologically relevant increases in micronucleated binucleated cells at concentrations evaluated (up to 175 µg/mL). In the MLA, mutant frequencies at all evaluable doses remained at or below concurrent control means and did not exceed the OECD Global Evaluation Factor (GEF); RTG and colony-size distributions were consistent with valid assay performance.

**Conclusions:** Under the applied test conditions, this *A. calamus* rhizome oil sample showed no evidence of direct mutagenic, clastogenic or aneugenic activity in a validated, complementary *in vitro* test battery. However, because (i) asarone isomers can be bioactivated via hepatic pathways not fully recapitulated *in vitro*, (ii) the study represents a single batch/chemotype with high β-asarone content, and (iii) *in vitro* cytotoxicity limited the top dose in some assays, we recommend targeted follow-up: chemotype variability screening, human-relevant metabolic studies, and *in vivo* genotoxicity with TK measurements to define systemic exposures and refine risk assessment.

## Keywords

*Acorus calamus,* genotoxicity, β-asarone, micronucleus test, Ames test, herbal safety, *in vitro* toxicology.