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Assessment of the genotoxic potential of mintlactone

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ABSTRACT

Mintlactone (chemical name 3,6-dimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one, CAS Number 13341-72-5) is a fragrance and flavor ingredient with reported uses in many different cosmetics, personal care, and household products. In order to evaluate the genotoxic potential of mintlactone, *in vitro* and *in vivo* genotoxicity tests were conducted. Results from bacterial mutagenicity tests varied across different batches of differing purity with positive results observed in TA98 only. An *in vivo* comet assay was also considered to be positive in livers of female mice but negative in male mice. In contrast, *in vitro* and *in vivo* micronucleus tests, as well as 3D skin comet/micronucleus tests, were negative, indicating no chromosomal or DNA damage. The underlying causes for these contradictory results are not clear. It appears that the purity and/or stability of the test material may be an issue. In the absence of dependable scientific information on the purity and/or storage stability of mintlactone, its safety for use as a fragrance ingredient cannot be substantiated.

1. Introduction

Mintlactone (chemical name 3,6-dimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one, CAS Number 13341-72-5) is a fragrance ingredient used in a range of cosmetics, personal care, and household products. Mintlactone has molecular weight of 166 Da, vapor pressure of 0.00167 mmHg, water solubility of 725.4 mg/L and Log $K_{o/w}$ of 2.28 (EPISuite). The total chronic systemic exposure through use as a fragrance ingredient (dermal, oral, and inhalation) is reported as 0.11 μg/kg bw/day (RIFM, 2020). While the systemic exposure estimate to mintlactone as a fragrance ingredient is below the threshold of toxicological concern (TTC) for a class III material (Munro et al., 1996a, 1996b, 2008; Kroes et al., 2004, 2007; Patel et al., 2020; Yang et al., 2017), the total chronic systemic exposure estimate exceeds the TTC for DNA reactive mutagens and/or carcinogens of 0.0025 μg/kg/day (More, 2019). Therefore, this material was prioritized for genotoxicity testing as part of the fragrance industry's ongoing ingredient review and assessment program (Api, 2015).

The aim of this paper is to report on the actions taken by the Research Institute for Fragrance Materials, Inc. (RIFM) to assess mintlactone and provide a consolidated report of the genotoxicity data collected on the material.

2. Methods and materials

2.1. Samples of mintlactone tested

Four different samples of Mintlactone (CAS Number 13341-72-5), from 2 different suppliers, were used across the different teststests described in this paper. Two samples with a stated purity of 96.9% (wt/wt) (Batch/Lot #1030003) and 97.1% (wt/wt) (Batch/Lot #10300013), stated respectively, were supplied by Symrise AG (Holzminden, Germany). Two samples each of 99.9% (wt/wt) purity (Batch # MKBZ8423V and Batch # MKCG4700), were purchased from Millipore Sigma at two different time points (Table 1). The majority of samples were conducted with Mintlactone Sample 1 (96.90% (wt/wt) content of Mintlactone).

All test samples were sent directly to the test laboratories by the suppliers. All samples were stored at room temperature and protected

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List of abbreviations

APC –	Aphidicolin
Bw	Body weight
B(a)P	Benzo(a)pyrene
BN	Binucleated cells
CBPI	Cytokinesis-Block Proliferation Index
CP	Cyclophosphamide
DMSO	Dimethylsulfoxide
DRF –	Dose-range finding
GLP –	Good laboratory practice
HPBL –	Human blood peripheral lymphocyte
MN	Micronuclei
MMC	Mitomycin C
MMS	Methyl methanesulfonate
MNBN	Micronucleated binucleated cells
MnRET	Micronucleated Reticulocytes
OECD	Organization for Economic Co-operation and Development
RET	Reticulocytes
RSMN –	Reconstructed skin micronucleus
RVCC	Relative vehicle cell count
S9 –	Rat liver postmitochondrial (S9) fraction (includes Phase I & II metabolic enzymes)
SD –	Standard deviation
TTC –	Threshold of toxicological concern
VB	Vinblastine

from light.

2.2. TestTest guidelines and GLP

All teststests were performed under Good Laboratory Practices (GLP) according to the United States Food and Drug Administration (US FDA) Good Laboratory Practices 21 CFR Part 58 and the Organization for Economic Co-operation and Development (OECD) Principles on Good Laboratory Practice ENV/MC/CHEM (98) 17 (Revised in 1997; issued January 1998).

Prescreening BlueScreen™ tests were conducted according to the methods described in (Hughes et al., 2012)

All other testtest protocols followed OECD Test Guidelines 471 (Bacterial reverse mutation assay), 487 *(in-vitro* micronucleus assay), 474 (*In vivo* micronucleus assay) and 489 (alkaline comet assay), respectively.

2.3. In vitro tests

2.3.1. Bacterial reverse mutation (Ames) assay

2.3.1.1. Test substance and control substances. Ames mutagenicity tests were conducted using the 4 different samples of mintlactone listed in Table 1. Test solutions were prepared in DMSO (purity >99.7%, Sigma Aldrich) freshly before incubation at the required concentrations. Positive control compounds (Benzo(a)pyrene and 2-aminoanthracene) were purchased from Sigma Aldrich (St. Louis, MO) for all the tests.

2.3.1.2. Test design. Mintlactone samples were evaluated with and without metabolic activation. Independent confirmatory assays were performed in all 5 tester strains at different concentrations in the presence and absence of rodent liver S9 fraction (Molecular Toxicology Inc., USA). Positive and vehicle controls were evaluated concurrently, and all test and control articles were evaluated in triplicate. Tester strains were exposed to mintlactone via plate incorporation by incubating tester strain, test article, and S9 mix (where appropriate) in molten top agar, which was subsequently overlaid onto a minimal bottom agar plate. (Ames et al., 1975; Maron and Ames, 1983). Following incubation, revertant colonies were counted(OECD, 1991).

2.3.2. In vitro micronucleus assay

2.3.2.1. Test substance and control substances. Mintlactone Sample 1 (96.90%, Table 1) was tested in this test. Positive controls were Cyclophosphamide (CP) and Vinblastine (VB) from Sigma Aldrich (St. Louis, MO).

2.3.2.2. Test design. Human peripheral blood lymphocytes (HPBL) were treated for 4 h in the absence and presence of S9 and for 24 h in the absence of S9 fraction. The test was conducted at BioReliance Laboratories Ltd, Rockville, Maryland (Dutta, 2018).

2.3.3. 3D skin comet

2.3.3.1. Test substance and control substances. Mintlactone Sample 1 (96.90%, Table 1) test solutions were prepared freshly in acetone to the required test concentrations. Positive controls, benzo(a)pyrene and methyl methanesulfonate [MMS] (Sigma Aldrich, St. Louis, MO USA), were dissolved in acetone (99.8% wt/wt) The Phenion Full-Thickness 3D Human Skin Model used in this assay was provided by Henkel (Düsseldorf, Germany).

2.3.3.2. Test design. The assay design was based on standards recommended by international expert groups for *in vitro* and *in vivo* comet procedures (Hartmann, 2003; Tice, 2000), and procedures in the OECD Test Guideline for the *in vivo* mammalian alkaline comet assay (OECD 489, 2016). 3D human skin tissues were treated with Mintlactone Sample 1 in acetone for 3 days (0, 24, and 45 h ± 30 min) by application of the test solution (25 μL) on the tissue surface. A preliminary toxicity test was conducted by exposing a single tissue per concentration to the vehicle alone and 11 concentrations (1.56–1600 μg/cm^2) of the test material. Post-treatment cytotoxicity (≥2-fold increase in adenylate

Table 1
Purities of Mintlactone used in the genotoxicity tests.

Chemical Name	Content of Mintlactone	Date Produced	Ames Test date	Source	Batch Number	Tests Conducted
Mintlactone Sample 1	96.90% (wt/wt)	March 11, 2014 (Test 1), July 2016 (Test 2)	May 27, 2014 (Test 1), January 02, 2018 (Test 2)	Symrise	1030003	Ames (Test 1 and 2), *in vitro* MNT, *in vivo* comet/MNT, 3Dskin comet/MNT
Mintlactone Sample 2	99.90% (wt/wt)	July 25, 2016	January 02, 2018	Sigma Aldrich	MKBZ8423V	Ames (Test 2)
Mintlactone Sample 3	99.90% (wt/wt)	May 21, 2018	June 18, 2020	Sigma Aldrich	MKCG4700	Ames (Test 3)
Mintlactone Sample 4	97.10% (wt/wt)	June 30, 2020	October 21, 2020	Symrise	10300013	Ames (Test 4)

kinase activity) was observed at concentrations ≥3.13 μg/cm^2. Based upon these results, the initial definitive comet assay was conducted using triplicate tissues at concentrations of 0.05–100 μg/cm^2. Cytotoxicity (≥2-fold increase in adenylate kinase activity) was observed at concentrations ≥25 μg/cm^2. The concentrations selected for the evaluation of comets were 1.0, 10, and 100 μg/cm^2. A confirmatory comet assay was conducted with a 3-day dosing regimen, as in the previous trials, at concentrations ranging from 0.05 to 100 μg/cm^2 using triplicate tissues. In addition, aphidicolin was added to the culture medium 4 h ± 15 min prior to harvest at 1 μL/mL (final concentration in medium of 5 μg/mL). Aphidicolin (APC), an inhibitor of DNA polymerases α and δ, was added into the protocol when the standard protocol did not show any effects. Inhibiting the DNA repair function of the polymerases by APC amplifies single-strand breaks generated during excision repair, which leads to increased comet formation, enhancing the sensitivity of the assay (Pfuhler et al., 2020). In the confirmatory comet assay, post-treatment cytotoxicity (≥2-fold increase in adenylate kinase activity or ≥50% reduction in ATP/protein ratio relative to the vehicle control) was not observed at any concentration. No visible precipitate was observed on top of the tissue at any concentration tested.

2.3.4. 3D skin micronucleus

2.3.4.1. Test substance and control substances. Mintlactone Sample 1 (96.90% (wt/wt), Table 1) test solutions were prepared freshly in acetone to the required test concentrations. Mitomycin C (Sigma Aldrich,St. Louis, MO,USA) was used as positive control; EpiDerm™ tissue (MatTek Corporation Ashland, MA, USA) was used in this experiment. The EpiDerm™ model consists of a multilayered, differentiated tissue of basal, spinous, granular, and cornified layers resembling the normal human epidermis (Curren et al., 2006). This system has been demonstrated to be sensitive to the clastogenic and aneugenic activity of a variety of chemicals (Curren et al., 2006).

2.3.4.2. Test design. Mintlactone Sample 1 was evaluated for its potential to induce micronuclei in the reconstructed skin micronucleus assay (RSMN) in EpiDerm™. Acetone was used as the vehicle. The preliminary toxicity test was conducted using a 2-day dosing regimen (48-h treatment) by exposing a single tissue per concentration to vehicle alone and 15 concentrations (0.0061–100 mg/mL) of the test substance. Cytotoxicity (≥50% cytokinesis-blocked proliferation index [CBPI] relative to the vehicle control) was observed at concentrations ≥12.5 mg/mL. Cytotoxicity (≥50% reduction in viable cell count [RVCC] relative to the vehicle control) was observed at concentrations ≥6.25 mg/mL. Based on these results, the micronucleus assay was conducted using triplicate tissues at concentrations ranging from 0.1 to 10 mg mintlactone/mL.

2.4. In vivo micronucleus and comet assays

2.4.1. Test substance and control substances

Mintlactone Sample 1 (96.90% (wt/wt), Table 1) test solutions were prepared freshly in corn oil to the required test concentrations. CP [for the micronucleus) and MMS [for the comet assay]) from ((Sigma Aldrich,St. Louis, MO,USA), dissolved in sodium chloride were used as positive controls. Hsd:ICR (CD- 1) mice were obtained from Envigo RMS Inc., Frederick, MD,USA.

2.4.2. Assay design

Mintlactone Sample 1 was applied via gavage to male and female Hsd:ICR (CD- 1) mice at 3 dose levels, (125, 250, and 500 mg/kg bw) on 3 consecutive days (0, 24, and 45 h before necropsy)(OECD, 1997, 2016a,b). These dose levels were selected based on a prior range-finder experiment to determinate the maximum tolerated dose. Peripheral blood was collected for micronucleus detection using flow cytometry. For the comet assay liver was selected as the site of metabolism to evaluate the potential genotoxicity of metabolites and as a tissue that reflects the bioavailability of a test substance. The comet assay was performed in whole cells isolated from liver tissues and not in isolated nuclei.

3. Results

3.1. Ames (bacterial reverse mutation) assays

3.1.1. Ames test 1

The mutagenicity of Mintlactone Sample 1 (96.90% (wt/wt), Table 1) was assessed using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA. The test material was cytotoxic at 5000 μg/plate in strain WP2uvrA in the presence and absence of S9 and in strains TA100 and TA1535. No precipitation was observed at any concentration in any strain. In tester strain TA98 in the absence of S9 (Fig. 1b), a ≥2-fold increase in the mean number of revertant colonies was observed at ≥1600 μg/plate in the initial mutagenicity assay and at ≥1000 μg/plate in the confirmatory mutagenicity assay.

A ≥2-fold increase in the mean number of revertant colonies was also observed in WP2uvrA at 5.00 and 50.0 μg/plate in the initial assay in the absence of S9 (Fig. 1j). This result, however, was deemed biologically nonrelevant and due to the low mean number of revertant colonies (6.0) in the concurrent vehicle control. Moreover, the mean number of revertant colonies at 5.00 and 50.0 μg/plate were 12.3 and 12.7, respectively, and were within the vehicle control historical range (8.00–28.0 mean number of revertant colonies) for this strain.

No increases in the mean number of revertant colonies were observed at any tested dose level in any other tester strain in both the initial and confirmatory assays in the presence or absence of S9 (Figure 1a, 1c-i). All positive and vehicle control values were within acceptable ranges, and all criteria for a valid test were met. Under the conditions of the test, Mintlactone Sample 1 was positive in TA98 in the absence of S9 but negative in all other tester strains in the presence and absence of S9 when tested up to 5000 μg/plate (Bhalli, 2017bl).

3.1.2. Ames test 2

In a repeat Ames test, the mutagenicity of Mintlactone Sample 1 (96.90%, Table 1) was re-assessed using *Salmonella typhimurium* strain TA98 and *Escherichia coli* strain WP2uvrA. The test material was cytotoxic at 5000 μg/plate in both strains TA98 and WP2uvrA in the absence of S9. No precipitation was observed at any concentration in any strain. All positive and vehicle control values were within acceptable ranges. A positive response (4.9-fold and 6.7-fold, maximum increase) outside of the 95%–99% historical control limits was observed with tester strain TA98 in the absence of S9 activation in the initial mutation assay and in the confirmatory mutagenicity assay, respectively (Fig. 2a). Under the conditions of the test, Mintlactone Sample 1 caused a positive response with tester strain TA98 in the absence of Aroclor-induced rat liver S9 activation (Dakoulas, 2020).

The mutagenicity of a second sample, Mintlactone Sample 2 (99.90% (wt/wt), Table 1), was also assessed under the same protocol using *Salmonella typhimurium* strain TA98 and *Escherichia coli* strain WP2uvrA. The test material was cytotoxic at 5000 μg/plate in strain WP2uvrA in the absence of S9. No precipitation was observed at any concentration in any strain. All positive and vehicle control values were within acceptable ranges. The test material did not induce any increases in the frequency of revertant mutations for any of the tester strains in the presence or absence of an S9 activation system (Fig. 2b and d). Under the conditions of the test Mintlactone Sample 2 (99.90% (wt/wt)) did not cause a mutagenic response with either of the tester strains (Dakoulas, 2020).

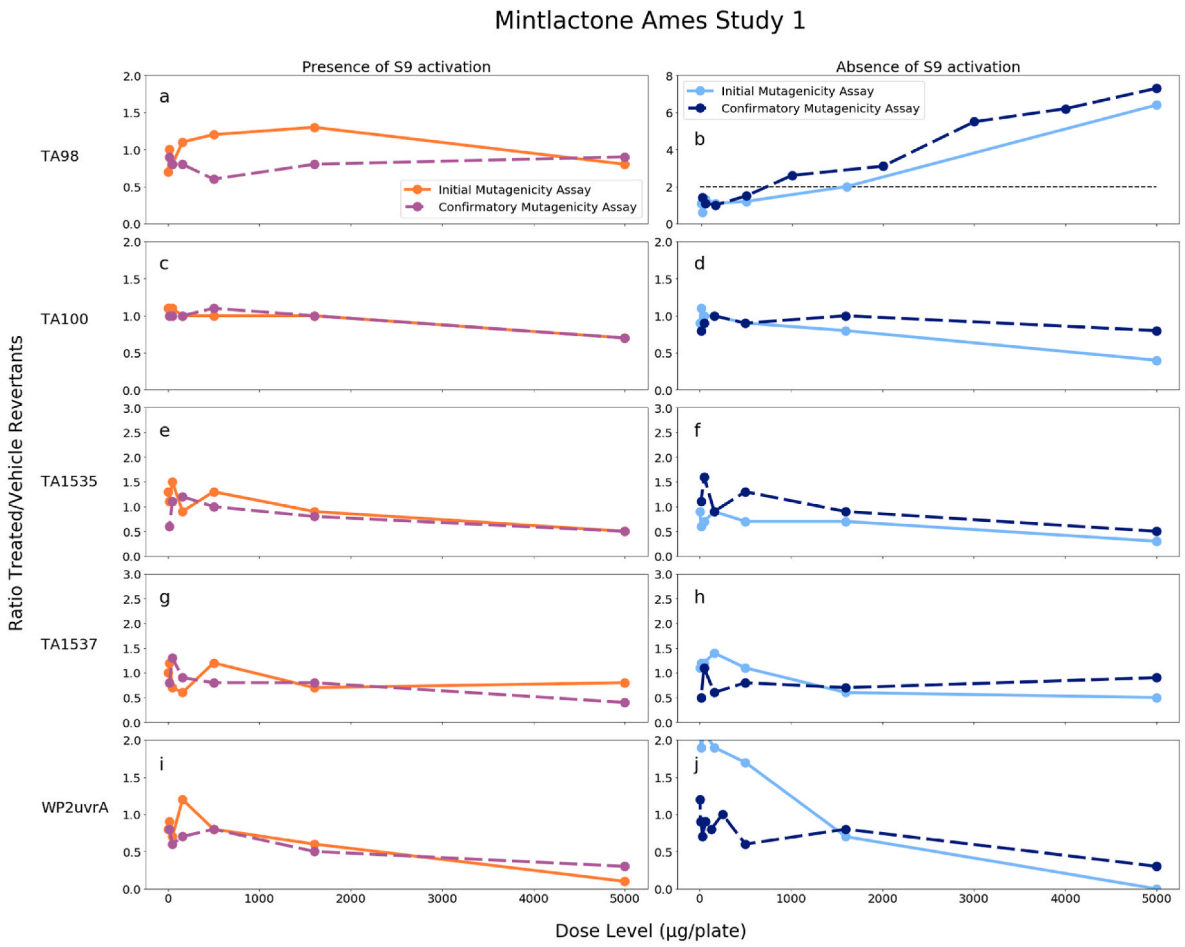

Fig. 1. Results of Ames Test Test 1 for Mintlactone Sample 1 (96.9% (wt/wt) content of mintlactone). *Dose levels (µg/plate) are shown compared to the ratio of the number of revertant colonies in the sample treated with Mintlactone Sample 1 compared to vehicle solvent control. The initial mutagenicity assay (bold; orange, light blue lines) and the confirmatory mutagenicity assay (dashed; purple, dark blue lines) are shown for each tester strain in the presence and absence of S9 activation. Values above a straight dashed line indicate a positive result. (a) TA98 in the presence of S9 activation, (b) TA98 in the absence of S9 activation, (c) TA100 in the presence of S9 activation, (d) TA100 in the absence of S9 activation, (e) TA1535 in the presence of S9 activation, (f) TA1535 in the absence of S9 activation, (g) TA1537 in the presence of S9 activation, (h) TA1537 in the absence of S9 activation, (i) WP2uvrA in the presence of S9 activation, (j) WP2uvrA in the absence of S9 activation. Positive mutagenic responses are observed when the ratio treated:vehicle revertants is greater than 2.0 in strains TA98, TA100, and WP2uvrA, or greater than 3.0 in strains TA1535 and TA1537. A positive mutagenic response can be observed in TA98 in the absence of S9 activation.*

3.1.3. Ames test 3

The mutagenicity of Mintlactone Sample 3 (99.90% (wt/wt), Table 1) was assessed using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA. The test material was cytotoxic at 5000 µg/plate in strain TA98 in the absence of S9 and in strain WP2uvrA in the presence and absence of S9. No precipitation was observed at any concentration in any strain. All positive and vehicle control values were within acceptable ranges.

In the initial mutation assay, a 2.1-fold and a 1.5-fold increase in the frequency of revertant mutations was observed in TA 98 and WP2uvrA, respectively, in the absence of S9. These increases were not dose-responsive and were within the historical control limits (Fig. 3b and j). In the confirmatory mutagenicity assay, positive responses (4.6-fold and 9.7-fold, respectively) were observed in strain TA98 in the presence and absence of S9 (Fig. 3a and b). Additionally, a 3.7-fold increase was observed in strain TA1537 in the absence of S9, and a 1.7-fold increase was observed in strain WP2uvrA in the presence of S9 (Fig. 3h and i). Both increases were not dose-responsive, but for TA1537, the increase was outside the historical control limit, and for WP2uvrA, the increase was at the upper end of the 99% historical control limit. Under the conditions of the test, Mintlactone Sample 3 caused a positive mutagenic response with tester strain TA98 in both the presence and absence of S9 (Dakoulas, 2020a).

3.1.4. Ames test 4

The mutagenicity of Mintlactone Sample 4 (97.10%, Table 1) was assessed using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA. The test material was cytotoxic at 5000 µg/plate in strain TA1537 in the presence of S9. The test material was cytotoxic at 3000 µg/plate in strain WP2uvrA in the presence and absence of S9. No precipitation was observed at any concentration in any strain. All positive and vehicle control values were within acceptable ranges.

In the initial mutation assay, a positive mutagenic response (2.4-fold increase) was observed in strain TA98 in the absence of metabolic activation, outside the historical control limit (Fig. 4b). A 2.9-fold increase was also observed in TA98 in the presence of S9, outside the historical control limit. Since it was not dose-responsive, it was considered equivocal (Fig. 4a). A 2.7-fold increase observed in strain TA1537 in the absence of S9, not dose responsive and with a vehicle control response at the lower end of the historical control range was considered not biologically relevant All other counts were within the 95% historical control limit (Fig. 4h).

In the confirmatory mutagenicity assay, a positive response (4.4-fold increase) was observed in strain TA98 in the absence of S9, outside the historical control (Fig. 4b). A 2.0-fold increase was observed in TA98 in the presence of S9, but all counts were within the 95% historical control

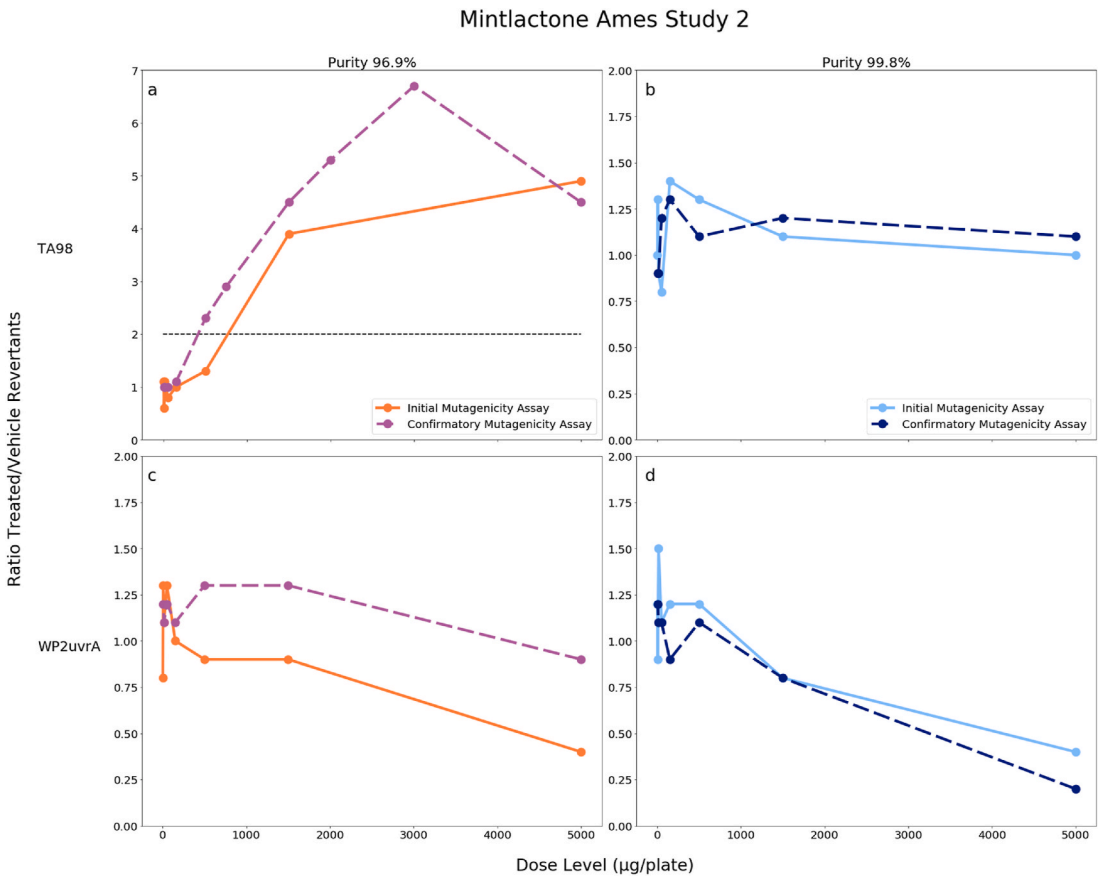

Fig. 2. Results of Ames Test Test 2 for Mintlactone Samples 1 and 2 (96.9% and 99.9% (wt/wt) content of mintlactone, respectively). *Dose levels (µg/plate) are shown compared to the ratio of the number of revertants in the sample treated with mintlactone to the sample treated with vehicle solvent. The initial mutagenicity assay (bold; orange, light blue) and the confirmatory mutagenicity assay (dashed; purple, dark blue) are shown for each tester strain in the absence of S9 when treated with either sample of mintlactone. Values above a straight dashed line indicate a positive result. (a) TA98 treated with sample 1, (b) TA98 treated with sample 2, (c) WP2uvrA treated with sample 1, (d) WP2uvrA treated with sample 2. Positive mutagenic responses are observed when the ratio of treated:vehicle revertants is greater than 2.0 in strains TA98 and WP2uvrA. A positive mutagenic response was observed in TA98 treated with sample 1.*

range and, therefore not considered biologically relevant (Fig. 4a). A 4.0-fold increase was observed in strain TA1537 in the absence of S9, not dose-responsive, and with a vehicle response at the lower end of the historical control range. All other counts were within the 95% historical control limit (Fig. 4h). However, This response was considered equivocal since it replicated the initial mutation assay.

Under the conditions of the test, Mintlactone Sample 4 (97.10% (wt/wt), Table 1) did cause a positive response with tester strain TA98 in the absence of S9 activation and equivocal responses with tester strain TA98 in the presence of S9 activation and TA1537 in the absence of S9 activation (Dakoulas, 2020b).

3.2. *In vitro micronucleus test*

Mintlactone Sample 1 (96.90% (wt/wt); Table 1) was assessed in the *in vitro* mammalian cell micronucleus test using human peripheral blood lymphocytes. No precipitate was observed at the beginning or end of treatment at any tested concentration in any treatment group. In the 4-h treatment in the absence of S9, concentrations of 50, 700, and 1400 µg/mL produced 3%, 23%, and 54% cytotoxicity, respectively, and were selected for MN scoring (Table 2). In the 4-h treatment in the presence of S9, concentrations of 50, 700, and 1250 µg/mL produced 17%, 21%, and 50% cytotoxicity, respectively, and were selected for MN scoring (Table 3). In the 24-h treatment in the absence of S9, concentrations of 5, 50, and 135 µg/mL produced 13%, 28%, and 54% cytotoxicity, respectively, and were selected for MN scoring (Table 4).

The highest concentration analyzed under each treatment condition produced a 50%–60% reduction in CBPI, which met the maximum acceptable concentration as recommended by testing guidelines for this assay. The micronucleated binucleated cell frequencies of the vehicle and the positive controls fell within the acceptable ranges. Mintlactone Sample 1 did not induce micronucleated binucleated cells relative to the vehicle control at any concentration level in either the presence or absence of an S9 activation system. Based on these findings, Mintlactone Sample 1 did not induce micronuclei in the *in vitro* mammalian cell micronucleus test (Dutta, 2018).

3.3. *3D reconstructed skin assays*

3.3.1. *3D reconstructed skin micronucleus assay*

Mintlactone Sample 1 (96.90% (wt/wt), Table 1) was tested in a GLP-compliant 3D reconstructed skin micronucleus (RSMN) assay using EpiDerm tissues. The top concentration selected for scoring micronuclei, 6 mg/mL, produced 18.67% group mean cytotoxicity based on cytokinesis-block proliferation Index (CBPI) and 51.33% group mean cytotoxicity based on relative vehicle cell count (RVCC); this was within the recommended range of 55 ± 5% cytotoxicity (Table 5). Hence this concentration along with two lower concentrations (0.25 and 2 mg/mL) were included in the evaluation of micronuclei based on RVCC. 1000 binucleated cells per tissue were scored for the presence of micronuclei. In the initial micronucleus assay, the percentage of cells with micronucleated binucleated cells (MN) was not increased relative to the vehicle control at any concentration tested. In the confirmatory MN assay, the top concentration selected for scoring, 6 mg/mL, produced

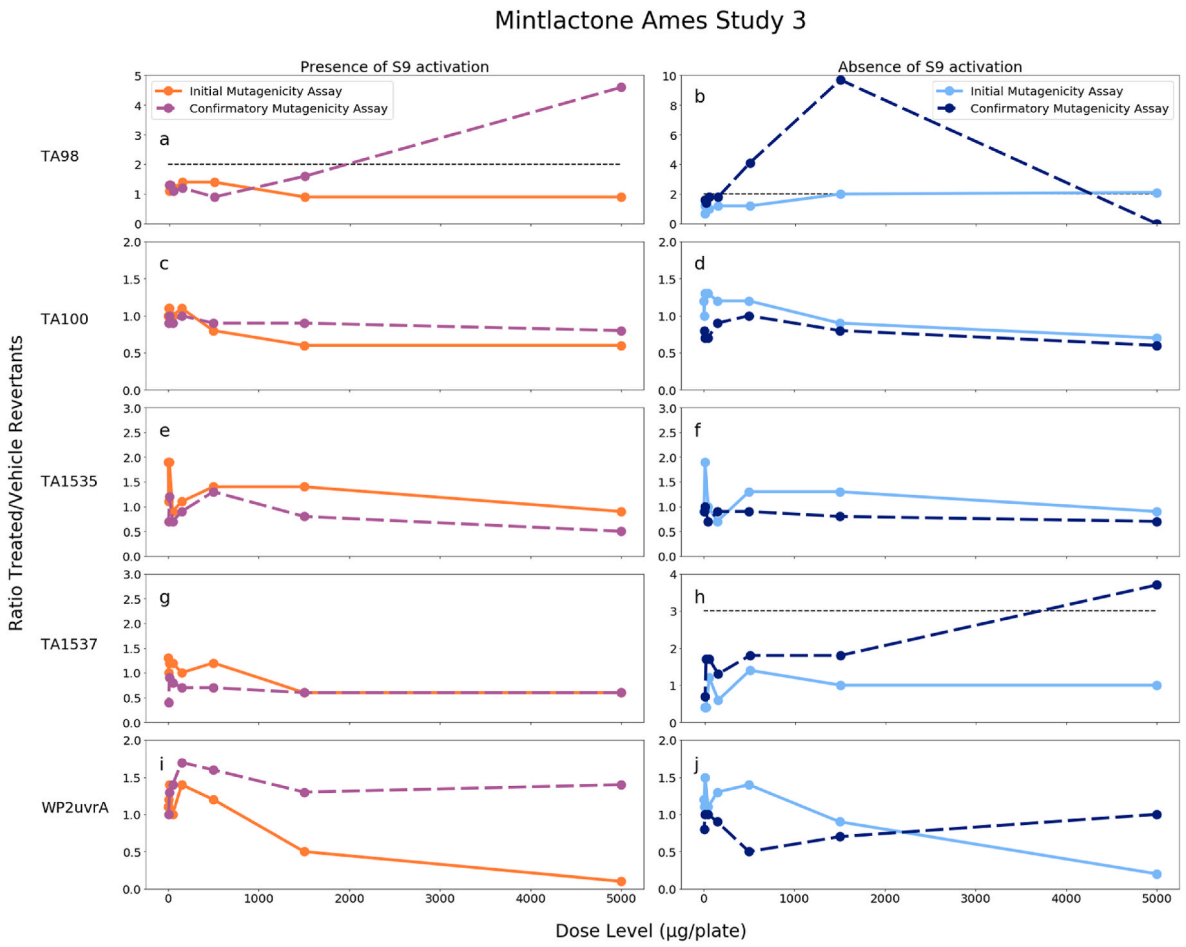

Fig. 3. Results of Ames Test Test 3 for Mintlactone Sample 3 (99.9% (wt/wt) content of mintlactone). *Dose levels (µg/plate) are shown compared to the ratio of the number of revertants in the sample treated with mintlactone to the sample treated with vehicle solvent. The initial mutagenicity assay (bold; orange, light blue) and the confirmatory mutagenicity assay (dashed; purple, dark blue) are shown for each tester strain in the presence and absence of S9 activation. Values above a straight dashed line indicate a positive result. (a) TA98 in the presence of S9 activation, (b) TA98 in the absence of S9 activation, (c) TA100 in the presence of S9 activation, (d) TA100 in the absence of S9 activation, (e) TA1535 in the presence of S9 activation, (f) TA1535 in the absence of S9 activation, (g) TA1537 in the presence of S9 activation, (h) TA1537 in the absence of S9 activation, (i) WP2uvrA in the presence of S9 activation, (j) WP2uvrA in the absence of S9 activation. Positive mutagenic responses are observed when the ratio treated:vehicle revertants is greater than 2.0 in strains TA98, TA100, and WP2uvrA, or greater than 3.0 in strains TA1535 and TA1537. A positive mutagenic response can be observed in TA98 in the presence and absence of S9 activation. The response in strain TA1537 in the absence of S9 activation was considered not biologically relevant as it was not dose-responsive or reproducible.*

14.67% group mean cytotoxicity based on CBPI and 51.00% group mean cytotoxicity based on RVCC (Table 6). Two further concentrations, 0.1 and 4 mg/mL, were included in the MN evaluation. The 6 mg/mL concentration was associated with 55 ± 5% cytotoxicity (RVCC). In the confirmatory MN assay, mintlactone did not increase the MN percentage relative to the vehicle control at any concentration tested. Based on these findings, Mintlactone Sample 1 was concluded to be negative for the induction of micronuclei in the RSMN in EpiDerm (Roy, 2020a).

3.3.2. 3D reconstructed skin comet assay

Mintlactone Sample 1 (96.90% (wt/wt), Table 1) was dissolved in acetone (99.8%) (Sigma aldrich) and evaluated for its genotoxic potential by the comet assay in reconstructed 3D human skin models. The test material gave a negative (non-DNA damaging) response in this assay in the dermis and epidermis of the Reconstructed 3D Human Skin Model. None of the test substance-treated slides showed significant increases in the % tail DNA compared to the vehicle control in the dermis and epidermis. The arithmetic mean of the solvent control did not exceed 20% tail intensity in both the dermis and epidermis and the positive control induced at least a 2-fold increase in % tail intensity and an absolute difference of at least 15% (MMS) or 5% [B(a)P], compared to the vehicle control in both the dermis and epidermis. Since the result of the definitive comet assay was negative, a confirmatory comet assay was conducted with a 3-day dosing regimen, as in the previous trials, at concentrations ranging from 0.05 to 100 µg/cm^2 using triplicate tissues. Mintlactone gave a negative (non-DNA damaging) response in this assay in the dermis of the Reconstructed 3D Human Skin Model. None of the treatment slides showed significant increases in the % tail DNA compared to the vehicle control in the dermis. The arithmetic mean of the solvent control did not exceed 20% tail intensity in both the dermis and epidermis and the positive control induced at least a 2-fold increase in % tail intensity and an absolute difference of at least 15% (MMS) or 5% [B(a)P] compared to the vehicle control in both the dermis and epidermis. Thus, the mintlactone associated increases were within the vehicle historical control range and hence considered to be biologically non-relevant. In conclusion, Mintlactone Sample 1 was considered negative in the *in vitro* 3D skin comet assay (Roy, 2020e) (see Table 7 and 8).

3.4. In vivo comet/micronucleus test

The DNA and chromosome damaging activity of Mintlactone Sample 1 (96.90% (wt/wt), Table 1) was evaluated in a combined *in vivo* comet/micronucleus test conducted in compliance with GLP regulations and in

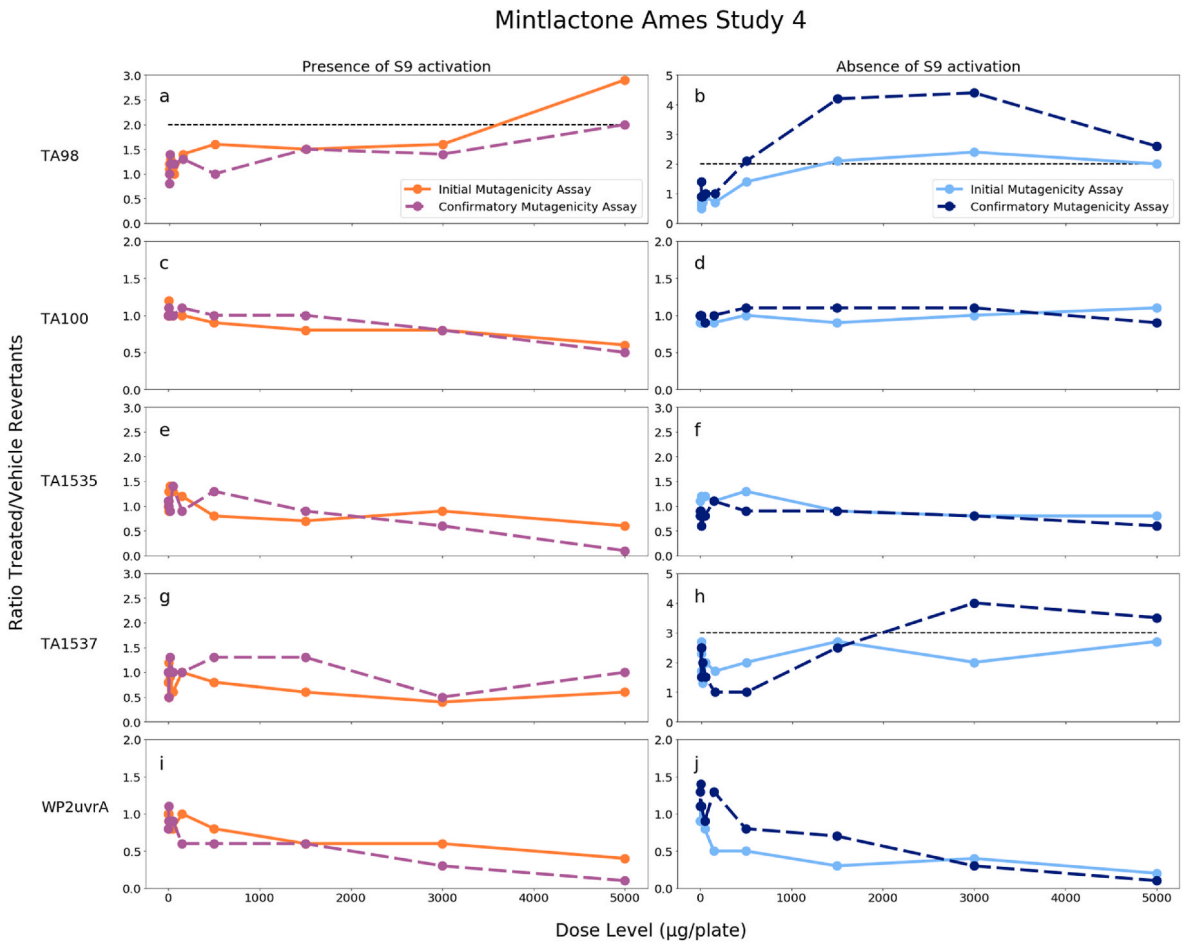

Fig. 4. Results of Ames Test Test 4 for Mintlactone Sample 4 (97.1% (wt/wt) content of mintlactone). *Dose levels (µg/plate) are shown compared to the ratio of the number of revertants in the sample treated with mintlactone to the sample treated with vehicle solvent. The initial mutagenicity assay (bold; orange, light blue) and the confirmatory mutagenicity assay (dashed; purple, dark blue) are shown for each tester strain in the presence and absence of S9 activation. Values above a straight dashed line indicate a positive result. (a) TA98 in the presence of S9 activation, (b) TA98 in the absence of S9 activation, (c) TA100 in the presence of S9 activation, (d) TA100 in the absence of S9 activation, (e) TA1535 in the presence of S9 activation, (f) TA1535 in the absence of S9 activation, (g) TA1537 in the presence of S9 activation, (h) TA1537 in the absence of S9 activation, (i) WP2uvrA in the presence of S9 activation, (j) WP2uvrA in the absence of S9 activation. Positive responses are observed when the ratio of treated:vehicle revertants is greater than 2.0 in strains TA98, TA100, and WP2uvrA, or greater than 3.0 in strains TA1535 and TA1537. A positive response can be observed in TA98 in the absence of S9 activation. Equivocal responses were observed in strains TA98 in the presence of S9 activation and TA1537 in the absence of S9 activation.*

Table 2
In vitro micronucleus analysis of HPBL treated with mintlactone sample 1 (96.90% (wt/wt)) in the absence of exogenous metabolic activation: 4-Hour treatment, 24-HOUR harvest.

Treatment (µg/mL)	Replicate Culture	Total Number of Cells Counted	CBPI[1]	Cytotoxicity[2]	Percentage of Micronucleated Binucleated Cells per Culture	Average Percent Micronucleated Binucleated Cells Per Concentration
DMSO	A	1000	1.527		0.60%	0.65%
	B	1000			0.70%	
Mintlactone						
50	A	1000	1.513	3%	0.60%	0.70%
	B	1000			0.80%	
700	A	1000	1.407	23%	0.40%	0.45%
	B	1000			0.50%	
1400	A	1000	1.244	54%	0.60%	0.55%
	B	1000			0.50%	

[1] CBPI = Cytokinesis-Block Proliferation Index.
[2] Relative to vehicle control.

accordance with OECD Guidelines. In the dose-range finding (DRF) test, mortality was observed in 3/3 male and 3/3 female mice receiving a dose of 1000 and 2000 mg/kg bw. Clinical signs following administration of 500 mg/kg bw included: piloerection and lethargy in males and piloerection, lethargy, and hunched posture in females. At higher dosage, clinical signs included: piloerection, lethargy, and convulsions in males and piloerection and lethargy in females. In the definitive assay, doses of 125, 250, or 500 mg/kgbw/day were administered. The test material did not induce a biologically relevant increase in the incidence of MnRETs (Table 9). In the comet assay, a statistically significant response at all concentrations was observed in liver cells of male mice and at the mid and high concentrations in female mice (Table 10 and

Table 3
In vitro micronucleus analysis of HPBL treated with mintlactone sample 1 (96.90% (wt/wt)) in the presence of exogenous metabolic activation: 4-Hour treatment, 24-HOUR harvest.

Treatment (μg/mL)	Replicate Culture	Total Number of Cells Counted	CBPI[1]	Cytotoxicity[2]	Percentage of Micronucleated Binucleated Cells per Culture	Average Percent Micronucleated Binucleated Cells per Dose
DMSO	A	1000	1.507		0.40%	0.45%
	B	1000			0.40%	
Mintlactone						
50	A	1000	1.419	17%	0.80%	0.65%
	B	1000			0.50%	
700	A	1000	1.401	21%	0.20%	0.30%
	B	1000			0.40%	
1250	A	1000	1.255	50%	0.30%	0.40%
	B	1000			0.50%	
CP[3]						
2.5	A	1000	1.614	27%	1.10%	1.15%**
	B	1000			1.20%	

** $p \leq 0.01$, Fisher's exact test, relative to the solvent control.
[1] CBPI = Cytokinesis-Block Proliferation Index.
[2] Relative to vehicle control.
[3] CP = Cyclophosphamide.

Table 4
In vitro micronucleus analysis of HPBL treated with mintlactone sample 1 (96.90% (wt/wt)) in the absence of exogenous metabolic activation: 24-Hour treatment, 24-HOUR harvest.

Treatment (μg/mL)	Replicate Culture	Total Number of Cells Counted	CBPI[1]	Cytotoxicity[2]	Percentage of Micronucleated Binucleated Cells per Culture	Average Percent Micronucleated Binucleated Cells per Dose
DMSO	C	1000	1.846		0.60%	0.50%
	D	1000			0.40%	
Mintlactone						
5	C	1000	1.734	13%	0.60%	0.80%
	D	1000			1.00%	
50	C	1000	1.610	28%	0.60%	0.55%
	D	1000			0.50%	
135	C	1000	1.387	54%	0.80%	0.70%
	D	1000			0.60%	
VB[3]						
10 ng/mL	C	1000	1.614	27%	2.10%	1.95%**
	D	1000			1.80%	

** $p \leq 0.01$, Fisher's exact test, relative to the solvent control.
[1] CBPI = Cytokinesis-Block Proliferation Index.
[2] Relative to vehicle control.
[3] VB = Vinblastine.

11). Dose dependence was observed at all concentrations in both male and female mice. Therefore, Mintlactone Sample 1 was concluded to be negative in the *in vivo* micronucleus test, however positive in the *in vivo* comet assay (Pant, 2020).

4. Discussion

Mintlactone was tested in the Ames test in 5 different experiments. Three different qualities of mintlactone were obtained from 2 suppliers. The first Ames test was conducted using a sample with 96.9% (wt/wt) purity (Mintlactone Sample 1) and was concluded to be positive in the TA98 strain in the absence of S9. A ≥2-fold increase in the mean number of revertant colonies was observed at ≥1600 μg/plate in the initial mutagenicity assay and at ≥1000 μg/plate in the confirmatory mutagenicity assay (Bhalli, 2017bl).

As a follow-up to the initial Ames positive tests, the material was also assessed in an *in vivo* comet/micronucleus assay. Mintlactone was administered at doses of 250 and 500 mg/kg/bw by gavage to CD-1 mice and the livers were analyzed for DNA breaks. There was no significant increase in the incidences of micronuclei. However, a positive response was observed in both male and female mice in the comet assay at the given doses of mintlactone. Significant dose-dependent increases were observed in the percent tail DNA in mintlactone dosed animals, exceeding the 95% control limit of the historical vehicle control. Histopathological analysis revealed centrilobular hypertrophy in male mice at doses of 250 and 500 mg/kg bw/day 2 animals with moderate and 1 with marked centrilobular hypertrophy at 500 mg/kg bw/day. Centrilobular hypertrophy is considered an adaptive response exerted in the liver by a putatively toxic treatment. The positive comet assay response observed in males may therefore be considered secondary to this effect. In contrast, in females, the positive comet assay was concluded to be due to test material (Pant, 2020), potentially caused by a toxic impurity or degradation product. The structurally related compound and potential precursor menthofuran likewise has been reported to produce a positive outcome in the comet assay in female mice (EMA, 2014; EMA, 2016).

The synthesis of Mintlactone is achieved by first hydrogenating hydroxymenthofurolactone to give dihydrohydroxymenthofurolactone which through subsequent elimination of water yields the product (U.S. Patent office, 2003) (Fig. 5).

By virtue of its unsaturated gamma-lactone structure, mintlactone can be conceived to be susceptible to hydrolytic or oxidative decomposition, if not adequately protected. Although not directly comparable, its sensitivity towards structural deterioration may also be reflected by the multiple metabolic transformations it undergoes in mammalian organisms. Some pertinent metabolites with supposedly DNA- or protein-binding potential are shown in Fig. 6. However, it is not clear whether such transformations may also occur within degradative processes

Table 5
INITIAL MICRONUCLEUS ASSAY: MICRONUCLEUS EVALUATION IN EPIDERM™ 3D SKIN TREATED WITH MINTLACTONE SAMPLE 1 (96.90% (wt/wt) mintlactone content).

Treatment (mg/mL)	Tissue No.	% Cytotoxicity Based on		BN Counted	% MNBN	% MNBN per Dose	
		CBPI	RVCC			Mean	±SD
Acetone	1			1000	0.10		
	2			1000	0.10	0.07	0.06
	3			1000	0.00		
0.25	7	−11	5	1000	0.20		
	8	−3	−1	1000	0.10	0.13	0.06
	9	0	9	1000	0.10		
2	16	−8	38	1000	0.10		
	17	13	36	1000	0.20	0.17	0.06
	18	10	28	1000	0.20		
6	22	33	52	1000	0.10		
	23	11	50	1000	0.00	0.03	0.06
	24	12	52	1000	0.00		
MMC, 6 μg/mL	34	52	60	1000	1.00		
	35	42	54	1000	0.80	1.03	0.25**
	36	49	52	1000	1.30		

BN = Binucleated cells.
MNBN = Micronucleated binucleated cells.
SD=Standard Deviation.
CBPI = Cytokinesis-Block Proliferation Index.
RVCC = relative vehicle cell count.
MMC = Mitomycin C.
** $p \leq 0.01$, Fisher's Exact Test, relative to the vehicle control.

Table 6
CONFIRMATORY ASSAY: MICRONUCLEUS EVALUATION IN EPIDERM™ 3D SKIN TREATED WITH MINTLACTONE SAMPLE 1 (96.90% (wt/wt) mintlactone content).

Treatment (mg/mL)	Tissue No.	% Cytotoxicity Based on		BN Counted	% MNBN	% MNBN per Dose	
		CBPI	RVCC			Mean	±SD
Acetone	1			1000	0.00		
	2			1000	0.20	0.10	0.10
	3			1000	0.10		
0.1	4	5	1	1000	0.10		
	5	3	10	1000	0.10	0.10	0.00
	6	−6	8	1000	0.10		
4	16	8	52	1000	0.00		
	17	8	54	1000	0.00	0.03	0.06
	18	10	52	1000	0.10		
6	22	16	49	1000	0.10		
	23	14	52	1000	0.00	0.10	0.10
	24	14	52	1000	0.20		
MMC, 5 μg/mL	31	13	52	1000	3.40		
	32	21	54	1000	3.20	2.73	0.99**
	33	18	49	1000	1.60		

BN = Binucleated cells.
MNBN = Micronucleated binucleated cells.
SD=Standard Deviation.
CBPI = Cytokinesis-Block Proliferation Index.
RVCC = relative vehicle cell count.
MMC = Mitomycin C.
** $p \leq 0.01$, Fisher's Exact Test, relative to the vehicle control.

during its storage.

The difference between samples 2 and 3 is the storage time before testing. Mintlactone Sample 3 (99.90% (wt/wt)) was manufactured in 2016, and the test was conducted in 2020, prior to batch expiration. This sample produced positive results in the Ames test. Mintlactone Sample 2 (99.90% (wt/wt)) was tested in an abbreviated Ames test (only 2 tester strains) in the same year as it was manufactured and was negative (Dakoulas, 2020; Dakoulas, 2020a). These divergent results may be due to potential degradation during the storage of Mintlactone Sample 3 (99.90% (wt/wt)). Unfortunately, there are no analytical data to support this. Certificates of analysis did not report genotoxic impurities in any of the samples which were reportedly stored by the test laboratories at room temperature under protection from light, in accordance with instructions from the suppliers. Still, the issue of stability of Mintlactone for the reported shelf life is deemed relevant and it is concluded therefore that further testing is not warranted unless this is resolved.

An additional full Ames test conducted with Mintlactone Sample 4 (97.10% (wt/wt)) was also concluded to be positive in the TA98 strain in the absence of S9. A ≥2-fold increase in the mean number of revertant colonies was observed at ≥1500 μg/plate in the initial mutagenicity assay and at ≥500 μg/plate in the confirmatory mutagenicity assay (Dakoulas, 2020e). The results support the premise that an unresolved purity/stability issue of the mintlactone samples investigated may underlie the observed direct mutagenic and DNA damaging effects.

An *in vitro* human lymphocyte micronucleus test and an *in vivo* micronucleus test were also negative for mintlactone (sample 1, 96.90%

Table 7
INITIAL TEST: COMET ASSAY CONDUCTED USING THE FULL-THICKNESS 3D SKIN MODEL TREATED WITH MINTLACTONE SAMPLE 1 (96.90% (wt/wt) mintlactone content).

Treatment	Dose (μg/cm^2)	No. of Replicates	Group Mean % of Clouds	% Tail DNA[A] in Dermal Cells (Mean ± SD)	Group Mean % of Clouds	% Tail DNA[A] in Epidermal Cells (Mean ± SD)
Acetone	0	3	4.3	4.45 ± 3.29	5.0	8.32 ± 1.41
Mintlactone	1.0	3	3.3	1.32 ± 0.19	6.7	9.85 ± 8.59
	10	3	4.0	2.20 ± 1.14	5.0	8.12 ± 5.71
	100	3	2.7	4.52 ± 1.76	6.3	9.04 ± 5.34
MMS[B]	5	3	6.7	37.60* ± 2.64	6.3	44.9* ± 9.15

SD = Standard Deviation.
MMS = Methyl methanesulfonate.
*p < 0.05 (Student t-test); Statistically significant increase relative to the vehicle control.
[A] Mean of 3 replicate means of medians.
[B] Dosed once approximately 3 h prior to cell collection on test day 3.

Table 8
CONFIRMATORY TEST: COMET ASSAY CONDUCTED USING THE FULL THICKNESS 3D SKIN MODEL TREATED WITH MINTLACTONE SAMPLE 1 (96.90% (wt/wt) mintlactone content).

Treatment	Replicates	AK (Fold)	ATP/Protein (%)	Group Mean % of Clouds	% Tail DNA[A] in Dermal Cells (Mean ± SD)	Group Mean % of Clouds	% Tail DNA[A] in Epidermal Cells (Mean ± SD)
Acetone-APC	3	1.0	0	5.3	7.87 ± 2.34	9.7	7.71 ± 6.26
Acetone + APC	3	1.0	0	7.7	10.90 ± 5.91	15.7	6.97 ± 5.38
2.5 μg/cm^2 mintlactone + APC	3	1.0	−197.9	7.0	15.62 ± 2.43	19.0	13.47@ ± 5.84
10 μg/cm^2 mintlactone + APC	3	1.4	−202.7	7.3	13.51 ± 2.06	10.0	16.05@ ± 2.90
31.6 μg/cm^2 mintlactone + APC	3	1.2	−110.4	5.7	15.28 ± 4.41	11.7	18.36@ ± 1.65
12.5 μg/cm^2 B(a)P -APC	3	1.0	−27.5	6.0	21.34* ± 3.76	12.7	17.59* ± 0.49
12.5 μg/cm^2 B(a)P + APC	3	1.0	−127.9	65.0	41.05* ± 3.63	43.3	51.19* ± 4.88

APC = Aphidicolin.
B(a)P = Benzo(a)pyrene.
SD = Standard Deviation.
*p ≤ 0.05 (Student's t-test); Statistically significant increase relative to the vehicle control.
@ p ≤ 0.01 (regression analysis): Statistically significant relative to the vehicle control.
[A] Mean of 3 replicate means of medians.

Table 9
SUMMARY OF IN VIVO PERIPHERAL BLOOD MICRONUCLEUS ANALYSIS IN MICE TREATED WITH MINTLACTONE SAMPLE 1 (96.90% (wt/wt) mintlactone content).

Treatment	Gender	Time (Hrs)	Animals	RET (Mean ± SD)			Cytotoxicity (%)	MnRET (Mean ± SD)			Total Cells Scored MnRET	Total Cells Scored RET
Vehicle												
0 mg/kg/day	M	3–4	6	1.97	±	0.73	–	0.06	±	0.03	54	91692
0 mg/kg/day	F	3–4	6	1.35	±	0.30	–	0.03	±	0.02	29	85752
Menthofurolactone												
125 mg/kg/day	M	3–4	6	1.25	±	0.38	−37	0.04	±	0.02	35	80010
125 mg/kg/day	F	3–4	6	1.16	±	0.39	−14	0.04	±	0.02	33	79003
250 mg/kg/day	M	3–4	6	1.56	±	0.38	−21	0.05	±	0.02	43	88930
250 mg/kg/day	F	3–4	6	0.89	±	0.18**	−34	0.04	±	0.01	37	85000
500 mg/kg/day	M	3–4	4	1.01	±	0.61	−49	0.08	±	0.02	36	47845
500 mg/kg/day	F	3–4	3	0.50	±	0.09	−63	0.07	±	0.06**	13	27298
CP												
20 mg/kg/dose	M	3–4	3	6.86	±	5.38	248	0.34	±	0.21	158	44279
20 mg/kg/dose	F	3–4	3	0.51	±	0.23**	−62	0.15	±	0.04*	47	30776

Treatment	Gender	Time (Hrs)	Animals	PCE (%) (Mean ± SD)			MnPCE (Mean ± SD)			Total Cells Scored MnPCE	Total Cells Scored PCE
CP											
20 mg/kg/dose	M	3–4	3	2.67	±	1.50	247.97	±	142.55**	156	80

** p < 0.01 as compared to vehicle control (Flow) (T-Test).
PCE – Polychromatic Erythrocytes; MnPCE – Micronucleated Polychromatic Erythrocytes; M – Males; Hrs – Hours, CP – Cyclophosphamide.
*p < 0.05 or **p < 0.01, One-Way ANOVA with Post Hoc Dunnett's Test or T-Test.
3 - 4 Hrs MnPCE Male GLM P-value = 0.077, R-sqr = 31.01%.
3 - 4 Hrs MnPCE Female GLM P-value = 0.238, R-sqr = 21.47%.
RET –Reticulocytes; MnRET – Micronucleated Reticulocytes; M – Males; F – Females; Hrs – Hours, CP – Cyclophosphamide.

Table 10
IN VIVO % TAIL DNA IN LIVER CELLS FOLLOWING FOUR ADMINISTRATIONS OF MINTLACTONE SAMPLE 1 (96.90% (wt/wt) mintlactone content) IN MALE MICE (SAMPLES COLLECTED 3 TO 4 HOURS POST-LAST DOSE).

Treatment (10 mL/kg/treatment)	Number of Animals	Group Mean % of Clouds	Tail DNA (%)[A] Mean	±	SD.	
Vehicle Control: Corn Oil	6	1.0	0.02	±	0.02	
Test Article: Mintlactone 125 mg/kg/day	6	0.8	1.75	±	1.09	@#
250 mg/kg/day	6	1.7	4.73	±	2.97	@#
500 mg/kg/day	4	1.0	4.34	±	2.93	@#
Positive Control: MMS 40 mg/kg[B] + CP 20 mg/kg[C]	3	3.7	7.69	±	2.68	*

SD = Standard Deviation.
*p ≤ 0.05 (Student's t-test); Statistically significant increase relative to the vehicle control.
@ p ≤ 0.01 (regression analysis): Statistically significant relative to the vehicle control.
# p ≤ 0.05 (ANOVA, Dunnett's post hoc); Statistically significant increase relative to the vehicle control.
[A] Mean of 3, 4, or 6 animals means of medians.
[B] Methyl methanesulfonate (MMS), positive control for the comet assay, orally administered only once at 3–4 h prior to organ collection on day 4.
[c] Cyclophosphamide (CP), positive control for the micronucleus assay, orally administered on test days 1 and 2.

Table 11
IN VIVO % tail DNA in liver cells following Four administrations of mintlactone (sample 1, 96.90% (wt/wt)) in female mice (samples collected 3 to 4 hours post-last dose).

Treatment (10 mL/kg/treatment)	Number of Animals	Group Mean % of Clouds	Tail DNA (%)[A] Mean	±	SD	
Vehicle Control: Corn Oil	6	0.7	0.01	±	0.00	
Test Article: Menthofurolactone 125 mg/kg/day	6	0.3	1.66	±	0.79	@
250 mg/kg/day	6	1.7	4.61	±	1.90	@#
500 mg/kg/day	3	1.0	7.79	±	1.82	@#
Positive Control: MMS 40 mg/kg[B] + CP 20 mg/kg[C]	3	1.7	5.46	±	5.28	§

SD = Standard Deviation.
@ p ≤ 0.01 (regression analysis): Statistically significant relative to the vehicle control.
# p ≤ 0.05 (ANOVA, Dunnett's post hoc); Statistically significant increase relative to the vehicle control.
§ p ≤ 0.05 (Mann-Whitney test); Statistically significant increase relative to the vehicle control.
[A] Mean of 3, 4, or 6 animals means of medians.
[B] Methyl methanesulfonate (MMS), positive control for the comet assay, orally administered only once at 3–4 h prior to organ collection on day 4.
[c] Cyclophosphamide (CP), positive control for the micronucleus assay, orally administered on test days 1 and 2.

(wt/wt)) (Dutta, 2018; Pant, 2020). Moreover, a 3D skin comet assay and micronucleus test were also negative (Roy, 2020a). Hence, based on these tests, mintlactone may be concluded not to have chromosome-damaging potential.

In summary, the evidence from the bacterial mutagenicity tests indicates that certain samples of mintlactone show direct mutagenic effects in the Ames test using strain TA98. In contrast to the bacterial mutagenicity tests, *in vitro* and *in vivo* micronucleus tests, as well as 3D skin comet/micronucleus tests, were negative with mintlactone (sample 1, 96.90% (wt/wt)), not indicating chromosomal or genotoxic damage. In contrast, with this material, an *in vivo* comet assay was considered to be positive in the livers of female mice (Table 12).

Altogether, we suspect that these contradictory results appear to reflect as yet unresolved purity and/or stability issues of the test material. In the absence of conclusive information on the purity/storage stability of mintlactone, the material is not considered safe for use as a fragrance ingredient.

Disclosures

All authors were active participants in the design, data analysis and interpretation of all the experiments. Benjamin PC Smith was supported by a National Research Foundation Singapore Whitespace grant (grant

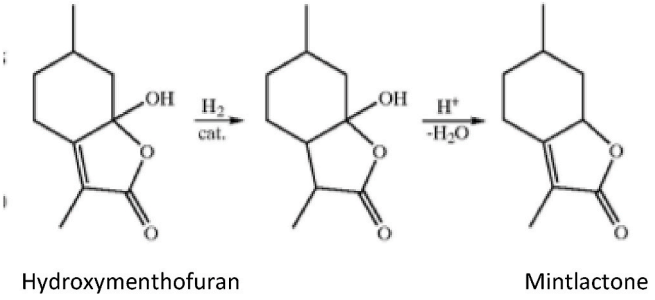

Fig. 5. Synthesis of mintlactone.

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CRediT authorship contribution statement

Yax Thakkar: Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments. **Holger Moustakas:** Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments.

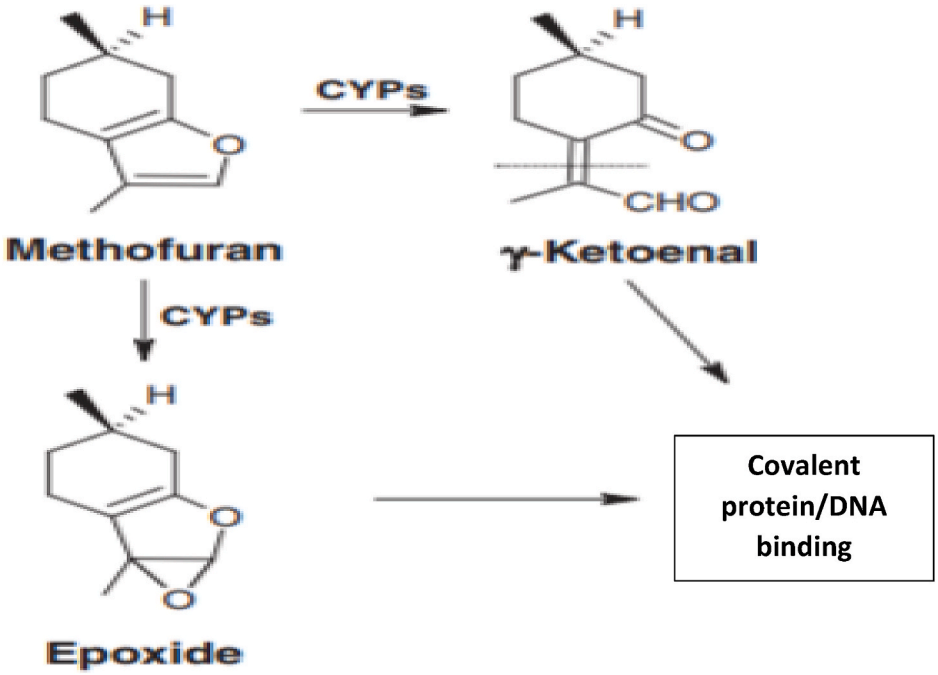

Fig. 6. Menthofuran metabolites which could potentially bind to DNA and protein (Modified from Chen et al., 2011).

Table 12
Summary table of mintlactone genotoxicity assays.

Sample number	DNA Damage			Mutagenicity	Chromosomal Damage		
	Screening BlueScreen	3D Skin Comet	In vivo Comet	Ames	In vitro MNT	3D Skin MNT	In vivo MNT
Mintlactone Sample 1 (96.90% (wt/wt))	Positive (- S9)	Negative	Positive (in liver) with histopathology	Positive in TA98 (-S9), questionable in WP2uvrA (- S9) Prescreening: Positive in TA98 (- S9) Prescreening: Negative in TA98 and WP2uvrA (- S9) Positive in TA98 (-/+S9), and TA1537 (- S9) Positive in TA98 (-S9), equivocal in TA1537 (S9)	Negative	Negative	Negative
Mintlactone Sample 2 (99.90% (wt/wt))	Positive (−/+ S9)						
Mintlactone Sample 3 (99.90% (wt/wt))							
Mintlactone Sample 4 (97.10% (wt/wt))							

Anne Marie Api: Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments. **Benjamin Smith:** Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments. **Gary Williams:** Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments. **Helmut Greim:** Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments. **Gerhard Eisenbrand:** Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments. **Wolfgang Dekant:** Data curation, All authors were active participants in the design, data analysis and interpretation of all the experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112659.

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