**ROBUST STUDY SUMMARIES:**
**Critical Studies Identified in Screening Assessment Targeted for Human Health.**

## 1.0 Chronic Toxicity/Carcinogenicity

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, US).

**METHOD:**

**Method/Guideline:** Other.
**Type of study:** 16-week toxicity study
**GLP:** Not stated.
**Year:** 1987
**Species and strain:** Rat, Sprague-Dawley
**No. of animals/dose:** 22 treated males/29 control males
**Route of exposure:** Oral (gavage)
**Vehicle:** 10% aqueous Emulphor EL-620
**Dose/Concentrations:** estimated to be 0 or 40 mg/kg-bw per day (0 or 1 mmol/kg bw per administration).
**Frequency and duration of exposure:** 3 times/week over 16 weeks.
**Duration of follow-up:** Animals killed by $CO_2$ asphyxiation at week 77..
**Analyses conducted:** Body weights, measured but frequency of measures not stated. Gross pathology and histopathology as per Rivenson et al. (1983).

**Statistical analysis:** Type of analyses and level of significance not stated. However, statistical significance stated for reported effects.

**RESULTS:**

**Food consumption:** Not stated.

**Mortality:** "After 16 weeks the dosing with 2-NP was discontinued due to several deaths in this group…"

**Clinical appearance:** Not stated.

**Body/organ weights:** "The body weights of rats gavaged with 2-NP were significantly lower than those of other groups throughout the study."

**Pathology:** Benign (4/22) and/or malignant (22/22; $p < 0.001$) liver tumours in treated rats. Control animals developed 1 benign and no malignant liver tumours. Lung metastases in 4/22, stomach papilloma in 1/22 and colon adenocarcinoma in 1/22 treated rats compared to none in the control rats.

**CONCLUSIONS:**

**2-Nitropropane is hepatocarcinogenic by the oral route in rats.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

Authors conducted the same study with 1-nitropropane, 1-azoxypropane, and 2-azoxypropane, but for these compounds, treatment was continued after 16 weeks with a different dosing regime (one gavage/week for 10 weeks). They stated that "The very high liver tumor incidence in the 2-NP group corroborates the results of a six month inhalation study at 207 p.p.m. reported by Lewis *et al.* [1979], and establishes that 2-NP is a strong carcinogen when given by gavage."

**REFERENCES:**

Fiala ES, Czerniak R, Castonguay A, Conaway CC, Rivenson A. 1987. Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity by gavage in Sprague-Dawley rats. Carcinogenesis 8: 1947–1949.

Rivenson A, Furuya K, Hecht SS, Hoffmann D. 1983. Experimental nasal cavity tumors induced by tobacco-specific nitrosamines (TSNA). In Reznik,G. and Stinson.S.F. (eds), *Nasal Tumors in Animals and Man, Vol. Ill, Experimental Nasal Cardnogenesis.* CRC Press, Inc., Boca Raton, FL, pp. 27-46.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** 94.5%

**METHOD:**

**Method/Guideline:** Other
**Type of study:** Inhalation study of 2 days, 10 days, 1 month, 3 months or 6-months duration.
**GLP:** No (conducted before implementation of GLP).
**Year:** 1979
**Species and strain:** Sprague-Dawley rat and New Zealand White rabbit.
**No. of animals/dose:** Rats - 10 males/group; Rabbits – 5 males/group
**Route of exposure:** Inhalation (whole body in 6 $m^3$ exposure chambers)
**Compound generation methods:** The substance was pumped at constant flowrate with either FMI pumps or a syringe drive to a vaporization apparatus. Vapourization was accomplished by utilizing a counter-current of heated air and the concentrated vapour-air mixtures were piped to the exposure chamber and diluted with the chamber airflow of approximately 1000 L/min. Infrared absorption spectrophotometry utilizing a Wilks MIRAN 1 was the analytical technique used. The MIRAN was connected to an automatic sampler so that the exposure chamber's concentration was monitored and calibrated every hour. Chamber concentrations were recorded twice per day.
**Dose/Concentrations:** 0, 27, or 207 ppm (equal to 0, 98 or 755 $mg/m^3$).
**Frequency and duration of exposure:** 7 hrs/day, 5 days/week.for all durations in rats; rabbits subjected to 1, 3, and 6 month durations.
**Duration of follow-up:** Animals were sacrificed by "overdose of pentobarbal" and exsanguinated.
**Analyses conducted:** Body weights, were measured biweekly. At necropsy, liver, kidneys, lungs plus trachea, brain and thyroid were weighed; adrenals, bronchi, cerebellum, cerebral hemispheres, eyes, kidneys, liver, lung, spleen, thyroid and trachea were fixed and stained for microscopic evaluation. Blood for haematological and serum biochemical analyses were collected from the abdominal aorta of rats and the marginal ear vein of rabbits. Evaluations for brain and lung edema were conducted using classical wet and dry weight techniques.

**Statistical analysis:** Rat data were analysed with Bartlett's test for homogeneity of variance followed by a 1-way analysis of variance; then analyzed by Student's t-test for inter-group differences. Rabbit data were analysed using the non-parametric Kruskal-Wallis 1-way analysis of variance; then analyzed by Mann-Whitney U test for inter-group differences. Testes weights were analyzed by covariance analysis, then by Student's t-test.

**RESULTS:**

**Chamber concentrations:** Overall exposure concentrations were 27.2 ± 4.6 ppm or 207 ± 15 ppm (equivalent to 99 ± 16.8 or 754 ± 54.7 mg/m$^3$, respectively).

**Mortality:** None

**Clinical appearance:** Not reported.

**Body weights:** Both rat and rabbit growth curves were similar to the control groups.

**Organ weights:** At 207 ppm, relative liver and lung weights in rats were significantly increased ($p < 0.005$) at 3 and 6 months.

**Haemotology and Serum biochemistry:** Decreased prothrombin times in both species at 1 and 3 months, but not at 6 months. Serum GPT (glutamic-pyruvic transaminase) levels [at 207 ppm?] were increased in rats at 10 days and 1 and 6 months (marked and indicative of liver cell damage at 6 months). At 207 ppm, serum OCT (ornithine carbamyl transferase) levels were increased in rabbits at 1 and 3 months but not at 6 months.

**Gross pathology:** At 207 ppm, pulmonary lesions (dark hemorrhagic foci scattered over all lobes) were observed in rats at 1, 3, and 6 months, and rat livers were paler in colour, showed surface lesions and areas of necrosis at 3 months. At 6 months at 207 ppm, rat livers were enlarged and pale with numerous masses and lesions. No effects observed in rabbits

**Pathology:** All rats exposed to 755 mg/m$^3$ for 6 months developed multiple hepatocellular carcinomas. Although there were no tumours seen in the rats exposed to 755 mg/m$^3$ for 3 months, non-neoplastic changes included hepatocellular hypertrophy (9/9 rats) and basophilic foci containing hyperplastic small hepatocytes with small hyperchromatic nuclei (4/9 rats). No tumours were noted in rats exposed to 98 mg/m$^3$. At 755 mg/m$^3$, 3/5 rabbits showed microscopic alterations (hemorrhage and congestion of alveoli and alveolar duct walls) in the lungs at 1 month, but not at 3 and 6 months. Evaluations of lung and brain edema were negative in both species.

Table 1. Incidence of liver pathology in rats exposed to 2-nitropropane for 6 months.

| Treatment group | Focal necrosis | Hepatocellular carcinoma | Neoplastic nodules |
|---|---|---|---|
| Control | 0/10 | 0/10 | 0/10 |
| 27 ppm | 1/10 | 0/10 | 0/10 |
| 207 ppm | 2/10 | 10/10 | 10/10 |

**CONCLUSIONS:**

**Potent liver carcinogen in the rat, but not a carcinogen in the rabbit.**
**NOAEL for non-neoplastic effects:** 98 mg/m$^3$.in both rats and rabbits.

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:** The authors conducted the same study using nitromethane at concentrations of 0, 98, or 745 ppm. For 2-nitropropane the authors stated, "Due to the findings of hepatocellular carcinomas and neoplastic nodules in rats, 2-NP should be handled in the workplace as if it were a human carcinogen."

**REFERENCE:**

Lewis TR, Ulrich CE, Busey WM. 1979. Subchronic inhalation toxicity of nitromethane and 2-nitropropane. J Environ Pathol Toxicol 2: 233–249.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** 95.7%

**METHOD:**

**Method/Guideline:** Other
**Type of study:** Inhalation study of 1, 3, 6, 12 or 22 month durations.
**GLP:** No.
**Year:** 1980
**Species and strain:** Sprague-Dawley rat (CRL: COBS CD SD BR).
**No. of animals/dose:** 125/sex/group
**Route of exposure:** Inhalation (whole body in a 21.75 $m^3$ exposure chamber)
**Compound generation methods:** 2-nitropropane vapours were generated by bubbling purified nitrogen through liquid 2-NP in an all-glass vessel maintained in a thermostatted waterbath at a temperature of 45°C. The concentration of 2-NP vapours was monitored by obtaining at least 3 samples/day from the exposure chamber using an air-sampling pump operating with a limiting orifice to control flow rate. Samples were analyzed with a gas chromatograph (flame ionization type).
**Dose/Concentrations:** 0, 25 ppm (equal to 0 or 78 $mg/m^3$).
**Frequency and duration of exposure:** 7 hrs/day, 5 days/week.for 22 months.
**Duration of follow-up:** Animals were sacrificed by "overdose of pentobarbal" and exsanguinated.
**Analyses conducted:** Animals were observed daily for clinical signs and body weights, were measured weekly. 10 rats/sex/group were killed after 1, 3, 6, and 12 months of exposure, and also at 3 and 12 months, 10 rats/sex were removed from the exposed group and remained under non-exposure conditions. All animals remaining alive were sacrificed at 22 months. At necropsy, body weights and liver, kidneys and brain were weighed. Skin and subcutis, mammary glands, spleen, lymph node, thymus, muscle, adipose tissue, kidney, urinary bladder, pituitary gland, adrenal gland, thyroid gland, trachea, lung, bronchus, heart, artery, prostate, seminal vesicle, testis, uterus, ovary, salivary gland, esophagus, stomach, small intestine, colon, mesentary, liver, pancreas, eye, ear, and brain.were fixed and stained for microscopic evaluation. Blood for haematological and serum biochemical analyses were collected from the aorta of rats.

**Statistical analysis:** "Where appropriate, Student's t test was used to compare treatment group means against the respective control group means."

**RESULTS:**

**Chamber concentrations:** Overall exposure concentration was 25.1 ± 1.4 ppm (equal to 78.3 ± 4.4 $mg/m^3$). As stated in Griffin et al. (1980), "The altitude of the site of the experiment is 1350 m, and at 25°C the concentration of 25 ppm is thus equivalent to 78 mg of 2-NP per cubic meter of air."

**Mortality:** Thirty-eight exposed males were found dead or sacrificed moribund compared to 21 controls over the 22 month period, but there was no difference in numbers of females found

dead or sacrificed moribund (36 exposed vs. 37 controls). The authors did not comment on mortality (numbers obtained from tables in Griffin et al. 1981).

**Clinical appearance:** No changes in appearance or behaviour.

**Body weights:** Body-weight gains were not affected. Final body-weights of females were significantly increased ($p < 0.01$) at the 6, 12, and 22 month sacrifices.

**Organ weights:** Relative liver weights in males were significantly increased at 6 ($p < 0.05$) and 22 months ($p < 0.01$). There were no significant differences in relative kidney or absolute brain weights.

**Haemotology and Serum biochemistry:** No consistent changes in measured parameters over the 22 month period.

**Gross pathology:** No changes.

**Pathology:** Liver congestion was slightly increased in both sexes (8/125 exposed vs. 1/125 control males and 7/124 exposed vs. 0/125 control females). Also observed were a slight increase in focal vacuolization of the cytoplasm of hepatocytes (58/125 exposed vs. 22/125 control males and 19/124 exposed vs. 18/125 control females) and in focal areas of hepatocellular nodules (10/125 exposed vs. 2/125 control males and 3/124 exposed vs. 1/125 control females). The distribution of tumours and other lesions in all organs examined, was similar between the control and exposed groups of rats.

**CONCLUSIONS:**

**LOAEL:** 78 $mg/m^3$ based on slightly increased focal vacuolization of the cytoplasm of hepatocytes and focal areas of hepatocellular nodules.

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

Griffin et al. (1980, 1981) suggested that the initial liver damage caused by 2-NP may lead to a physiological process of hyperregeneration during recovery phases of the experiment (which could be interpreted as a tissue process of hepatic regeneration leading to the normal physiologic state). Under conditions of continued daily, chemical (2-NP) exposure at concentrations far exceeding 25 ppm, it is not possible for the cellular regeneration process to overcome the insult to the cells, resulting potentially in a condition for any emerging hepatic cells to become autonomous further leading to neoplasia.

**REFERENCES:**

Griffin TB, Coulston F, Stein AA. 1980. Chronic inhalation exposure of rats to vapors of 2-nitropropane at 25 ppm. Ecotoxicol Environ Saf 4: 267–281.

Griffin TB, Stein AA, Coulston F. 1981. Histologic study of tissues and organs from rats exposed to vapors of 2-nitropropane at 25 ppm. Ecotoxicol Environ Saf 5: 194–201

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Sigma-Aldrich, Inc., Milwaukee, Wisconsin. US).

**METHOD:**

**Method/Guideline:** Other
**Type of study:** Oral study of 4 months.
**GLP:** Not stated.
**Year:** 2002
**Species and strain:** Rats, Eker strain (Long Evans background strain)
**No. of animals/dose:** 15 treated males and 10 control males.
**Route of exposure:** Oral (gavage)
**Vehicle:** 10% Emulphor
**Dose/Concentrations:** 89 mg/kg-bw, 3 days/week (equivalent to 38 mg/kg-bw per day); dose volume of 2 ml/kg bw.
**Frequency and duration of exposure:** 3 days/week (equivalent to 38 mg/kg-bw per day); for 4 months.
**Duration of follow-up:** Animals killed at end of study.
**Analyses conducted:** Necropsies performed on all animals and a complete set of tissues collected. Tissues were fixed and stained, and numbers of hyperplastic tubules, adenomas and carcinomas were counted in 2 different sections of the kidney for each animal. Gross renal masses were also counted and examined histologically. Non-renal neoplasms were also counted.

**Statistical analysis:** A Likelihood Ratio Test and a non-parametric Mann-Whitney U-test were reported as upper-tailed tests at 5% significance level.

**RESULTS:**

**Mortality:** None

**Pathology:** No consistent effect on numbers of preneoplastic or neoplastic renal lesions was observed in the exposed animals. Renal adenomas were non-significantly increased in the treated group (13/15 treated vs. 4/10 controls). Non-renal lesions included hepatocellular carcinoma (1/15 treated vs. 0/10 controls) and epididymal leiomyoma (1/15 treated vs. 0/10 controls), but these were non-significant. [No neoplasms in the expected target organ (liver) were found.]

**CONCLUSIONS:**

**NOAEL:** 38 mg/kg-bw per day.

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The purpose of the study was to examine the response of the Eker rat to nephrotoxic compounds and to genotoxic non-renal carcinogens. The same study was conducted with 2,4-diaminotoluene for 4 months and with cyclosporine, furan or aluminum-nitriloacetic acid for 4 or 6 months. The authors stated, "There were also no apparent qualitative or quantitative differences in the hepatic response of Eker rats, compared to wild-type Long Evans rats, treated with either 2,4-diaminotoluene (2,4-DAT) or 2-nitropropane (2-NP) for 4 months."

**REFERENCE:**

Morton LD, Youssef AF, Lloyd E, Kiorpes AL, Goldsworthy TL, Fort FL. 2002. Evaluation of carcinogenic responses in the Eker rat following short-term exposure to selected nephrotoxins and carcinogens. Toxicol Pathol 30(5): 559–564.

## 2.0 Genotoxicity

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated.(obtained from Aldrich Chemical Company, Milwaukee, Wisonsin, US)

**METHOD:**

**Method/Guideline:** Other.
**Type of study:** Gene mutation and DNA damage study.
**GLP:** Not stated.
**Year:** 2002.
**Species and strain:** Mice, C57BL/6 strain. DNA polymerase β heterozygous knockout mice (β-pol$^{+/-}$) were also produced and tested (40-50% reduction in mRNA and protein levels of DNA polymerase β**).**
Rats, F344 strain, also tested for p53 protein and β-pol protein levels
**No. of animals/sex/dose**: 3-4 male animals/group.
**Route of exposure:** Intraperitoneal injection
**Vehicle:** Olive oil
**Dose/Concentrations:** 100 mg/kg-bw.
**Frequency and duration of exposure:** One injection..
**Duration of follow-up:** Animals were killed after 24 hrs or after 2 wks. Tissues were flash frozen in liquid nitrogen.
**Analyses conducted:** Crude nuclear extract was obtained by homogenization of tissues, centrifugation, then precipation into a pellet which was dissolved and dialyzed with a dialysis buffer. Insoluble materials were removed by centrifugation and the supernatant was stored at -20°C for use in the repair assay and western blot analysis.
Western blot analysis: Nuclear extracts from livers of control and treated animals were subjected to SIDS-PAGE. Western blot analysis was accomplished by monoclonal anti-sera developed against mouse p53 and developed against rat β-pol. The bands were detected using an Alpha Innotech MultiImage™ system.
In vitro analysis of base-excision repair (BER): Radio-labeled oligonucleotides were incubated in a reaction mixture with nuclear extract from hepatocytes of control or treated animals. The oligonucleotides were extracted and precipitated and reaction products were visualized and quantified.

**Statistical analysis:** ANOVA used to determine significance between means, followed by Fisher's least significance test where appropriate. Differences between control and treated and/or between wild-type and knockout mice was analyzed by 2-factor analysis of variance followed by Sidak's multiple comparison test. $P < 0.01$ was considered to be statistically significant.

**RESULTS:**

**Analysis of DNA damage:** A significant increase in 8-hydroxydeoxyguanosine levels ($p < 0.01$), representing removal, i.e. DNA repair of this damaged base and an increase in levels of DNA

single strand breaks (4- to 5-fold), measured using the Comet assay were observed in the livers of treated animals. As an additional measure of damage induction, an increase in the protein level of the tumour suppressing gene *p53* ($p < 0.01$) was also observed from the nuclear extracts of both mice and rats.

**Analysis of mutant frequency:** In mice sacrificed 2 wks after exposure, an increase of mutant frequency in the *LacI* gene (2- to 3-fold) in the liver was observed, demonstrating that damage induced by 2-NP becomes fixed in the genome.

**Analysis of DNA repair activity in response to oxidative damage:** An in vitro G:U mismatch assay was used to measure BER activity. Nuclear extracts from the livers of treated mice showed significantly increased BER activity ($p < 0.01$).

**Analysis of β-pol protein levels in response to oxidative damage:** β-pol protein levels from nuclear extracts of livers from both mice and rats sacrificed after 24 hrs were significantly increased ($p < 0.01$), suggesting that DNA polymerase β (β-pol) is necessary for the repair of 2-NP oxidative damage.

**Analysis of the effects of oxidative damage in β-pol$^{+/-}$ mice:** When compared to the wild-type mice treated with 2-NP, β-pol$^{+/-}$ mice treated with 2-NP, nuclear extracts from hepatocytes showed significant increases in the level of DNA single strand breaks and in p53 protein levels. However, 8-hydroxydeoxyguanosine levels were similar, showing that the initial step of BER was properly regulated.

**CONCLUSIONS:**

**Gene mutation induced in mice via i.p. injection of 2-NP.**
**DNA damage induced in mice and rats via i.p. injection of 2-NP.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The purpose of the study was to determine the role of base-excision repair (BER) and DNA polymerase β (β-pol) in the removal of oxidized bases as well as determine the induction of BER and β-pol in vivo. 2-NP was selected to induce oxidative damage.

**REFERENCE:**

Cabelof DC, Raffoul JJ, Yanamadala S, Guo Z, Heydari AR. 2002. Induction of DNA polymerase beta-dependent base excision repair in response to oxidative stress *in vivo*. Carcinogenesis 23: 1419–1425.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Aldrich Chemical Company, Milwaukee, Wisonsin, US).

**METHOD:**

**Method/Guideline:** Other.
**Type of study:** Oxidative DNA damage study.

**GLP:** Not stated.
**Year:** 1998.
**Species and strain:** Rats, F344 strain.
**No. of animals/sex/dose:** Males, numbers not specified.
**Route of exposure:** Intraperitoneal injection, or oral (gavage) administration
**Vehicle:** Not stated.
**Dose/Concentrations:** 1 mmol/kg-bw (equivalent to 90 mg/kg-bw).
**Frequency and duration of exposure:** Not stated, but probably one injection or one dose only.
**Duration of follow-up:** Animals were killed after 18 hrs. The livers were immediately excised and rinsed in ice-cold 0.15 M NaCl and 0.015 M sodium citrate and frozen at -70 °C until the isolation of nucleic acids.

**Analyses conducted:** DNA and RNA were isolated and hydrolyzed with nuclease P1 and alkaline phosphatase at pH 5.4 and 7, respectively. The enzymatic hydrolysates were analyzed using reverse-phase HPLC (Beckman Ultrasphere ODS column with an Ultrasphere 5 $\mu$m, C18 guard column.

**Statistical analysis:** Not performed.

**RESULTS:**

The base moiety of the third unidentified nucleic acid modification in the liver RNA and DNA of 2-nitropropane treated rats is 2-hydrazinohypoanthine ($N^2$-aminoguanine).

**CONCLUSIONS:**

**2- nitropropane induced nucleic acid modifications in rat liver via intraperitoneal injection or oral administration.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The purpose of the study was to determine the base moiety of the unidentified third nucleic acid modification of RNA and DNA in the liver of 2-nitropropane treated rats. As proposed in the previous studies by Sosum and his colleagues, the formation of an unsubstituted nitronium ion, $NH_2^+$, as an ultimate reactive species in the activation pathway of 2-nitropropane, is capable of aminating proteins and amino acids. The authors believed that this activation pathway also applies to other carcinogenic secondary nitroalkanes such as 2-nitrobutane and 3-nitropentane that have been shown to be hepatocarcinogens in the rat model.

**REFERENCE:**

Sodum RS, Fiala ES. 1998. N2-amination of guanine to 2-hydrazinohypoxanthine, a novel in vivo nucleic acid modification produced by the hepatocarcinogen 2-nitropropane. Chem Res Toxicol 11: 1453–1459.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** 95% (obtained from Aldrich Chemical Company, Milwaukee, Wisonsin, US).

**METHOD:**

**Method/Guideline:** Other.
**Type of study:** DNA damage study.
**GLP:** Not stated.
**Year:** 1991.
**Species and strain:** Rats, Sprague-Dawley strain.
**No. of animals/sex/dose:** 4-12 animals/group, depending on dose levels and target organs.
**Route of exposure:** Oral (gavage) administration.
**Vehicle:** Not stated.
**Dose/Concentrations:** 0.5, 2 or 8 mmol/kg-bw (equivalent to 45, 180 or 720 mg/kg-bw).
**Frequency and duration of exposure:** Single dose
**Duration of follow-up:** Animals were killed after 1, 3, 6, 14 or 36 hrs. All subsequent steps were performed at 4 $^0$C. Livers, lungs, kidneys, brain and bone marrow were quickly removed and then separately processed in order to obtain suspensions of cells and/or nuclei.

**Analyses conducted:** Liver and kidney were minced and then incubated in Merchant's solution. Lung and brain suspensions were obtained by mincing of tissues on a stainless steel screen and by washing of cells through the screen with Merchant's solution. Bone marrow cells were obtained by dispersion of marrow plugs from femur into Merchant's solution. The alkaline elution was performed and the presence of DNA single-strand breaks and/or alkali labile sites were revealed by accelerated DNA elution as compared with controls. The content of DNA in the eluate and that remaining on the filter were determined by a micro fluorimetric procedure. Results were expressed both as percentage of DNA eluted from the filter and as Kt/Kc (relative elution rate) where Kt is the elution rate constant of DNA from treated rats and Kc is the elution rate constant of DNA from control rats. K was calculated from the equation K = (-InFR)/V, where FR is the eluting volume (13ml).

**Statistical analysis:** The nonparametric Wilcoxon two-sample two-tailed test was used. $P < 0.02$ was considered to be statistically significant.

**RESULTS:**

In the liver, DNA fragmentation was detected 1h after administration, reached its maximum after 6 h, and was partially repaired after 36 h; its amount was dose-depentent in the range of 0.5-8 mmol/kg. At 8 mmol/kg, there was no real evidence of DNA fragmentation in the bone marrow, lung, kidney and brain.

**CONCLUSIONS:**

**Oral administration of 2-nitropropane in rats produced DNA fragmentation in the liver where the damage was not completely repaired within 36 h. In contrast, DNA fragmentation was absent in lung, kidney, bone marrow and brain.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The findings from this study were consistent with the previously reported detection of DNA adducts, DNA repair synthesis and micronuclei formation in rat liver cells, as well as with the hepatocarcinogenicity of 2-nitropropane in the same species.

**REFERENCE:**

Robbiano L, Mattioli F, Brambilla G. 1991. DNA fragmentation by 2-nitropropane in rat tissues, and effects of the modulation of biotransformation processes. Cancer Lett 57: 61–66.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Waco Pure Industry Co., Osaka, Japan).

**METHOD:**

**Method/Guideline:** Other.
**Type of study:** Protective effect of green tea on oxidative DNA damage
**GLP:** Not stated.
**Year:** 1998.
**Species and strain:** Rats, F344 strain.
**No. of animals/sex/dose:** 5 male animals/group.
**Route of exposure:** Oral (gavage)
**Vehicle:** Distilled water containing 0.1% Tween 20.
**Dose/Concentrations:** 60 mg/kg bw,(low dose); or 90 mg/kg bw, followed by 120 mg/kg bw (high dose).
**Frequency and duration of exposure:** .Three times per week for 2 weeks for the low dose group; for the high dose group, two times of 90 mg/kg bw followed by four times of 120 mg/kg bw during a two week period.
**Duration of follow-up:** Animals were killed 4 hrs after the last treatment. Blood was collected from the orbital venous plexus, and the serum was prepared for measuring GOT and triglyceride levels. One part of the liver was immediately frozen for bio-chemical analysis and 8-OHdG level, and the other was fixed in ice-cold acetone for immunohistochemical analysis of BrdU, or in 10% formalin for haematoxylin and eosin (H&E) staining.

**Analyses conducted:**

**Biochemical analysis:** The lipid peroxidation level of the liver was determined as thiobarbituric acid reactive substances (TBARS) by the fluorometric method of Ohkawa et al. (1979), and expressed as the amount of malondialdehyde (MDA). The glycogen content of the liver was determined by the method of Seifter et al. (1950). GOT activity and levels of triglyceride in serum were measured using the kits (Test Wako).

**DNA extraction and measurement of 8-OHdG:** The livers were homogenized in phosphate buffered saline containing EDTA, and the nuclear fraction was obtained by centrifugation. This fraction was suspended in the same buffer and mixed with protenase K. Nuclear DNA was extracted with a DNA system and digested by treating with nuclease P1and alkaline phosphatase. The level of deoxyguanosine (dG) in the sample was determined by measuring its absorbance at 290 nm, and the level of 8-OHdG (8-OHdG /105 dG) was measured by an electrochemical connected to an HPLC.

**Analysis of cell proliferation**
To detect cells which had incorporated BrdU, routine sections were prepared and stained immuno-histochemically using an ABC kit. Cell proliferation was expressed a percentage value, the labelling index, derived from the number of labelled cells divided by the total number of cells counted (at least 3000 hepatocytes/rat).

**Statistical analysis:** The data were expressed as the mean $\pm$ SD of values for five rats per group; they were analysed for the significance of intergroup differences using Student's t-test. $P<0.05$ was considered to be statistically significant.

**RESULTS:**

**Biochemical indices:** There was a significant increase of 1.4-fold in serum GOT level, an index of injury to the liver, in rats treated with the high dose of 2-nitropropane. Changes in lipid peroxidation were measured in the liver as an index of oxidative stress. TBARS levels were elevated 1.6-fold in the low-dose group and 3.4-fold in the high-dose group. A dose-dependent decrease in the hepatic glycogen was detected in the 2-nitropropane treated groups. The level in the low-dose group was reduced to 55% of the control value, and that in the high-dose group was down to 38%. The serum triglyceride level was also significantly decreased in a dose-dependent manner by treatment, by 58% in the low-dose group and 85% in the high-dose group compared with the control group.

**Histopathological changes:** Histopathological examination revealed that 2-nitropropane treatment with low dose caused a slight swelling but no degenerative changes of hepatocytes. In the high-dose group, the hepatocytes were severely swollen, and degenerative changes, including single cell necrosis, were detected.

**8-OHdG formation in the liver:** There was a dose-related significant elevation in the hepatic 8-OHdG level with a 1.8-fold rise in the group treated with low-dose of 2-nitropropane, and a 2.9-fold rise in the high-dose group compared with the control level.

**Cell proliferation in the liver:** Cell proliferation in the liver after the 2-nitropropane treatment was determined by counting the relative number of hepatocytes that had incorporated BrdU, the so-called labelling index. There was a significant dose-related increase in labelling index of 2.5-fold in the low-dose group and 5.7-fold in the high-dose group compared with the control value.

**CONCLUSIONS:**

**This study demonstrated that the intake of green tea prevented hepatotoxicity, oxidative DNA damage and cell proliferation in the rat liver after repeated doses of 2-nitropropane.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The purpose of this study was to evaluate the benefit of green tea in mitigating hazards caused by repeated exposure of 2-nitropropane.

**REFERENCE:**

Sai K, Kai S, Umemura T, Tanimura A, Hasegawa R, Inoue T, Kurokawa Y. 1998. Protective effects of green tea on hepatotoxicity, oxidative DNA damage and cell proliferation in the rat liver induced by repeated oral administration of 2-nitropropane. Food Chem Toxicol 36: 1043–1051.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** 97% (laboratory grade, obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, US).

**METHOD:**

**Method/Guideline:** Other.
**Type of study:** Oxidative DNA damage study using 2-nitropropane as positive control
**GLP:** Not stated.
**Year:** 1999.
**Species and strain:** Rats, Fischer strain.
**No. of animals/sex/dose:** six male animals/group.
**Route of exposure:** Intrperitoneal injection
**Vehicle:** 1:4 v:v of Alkamuls:water.
**Dose/Concentrations:** 100 mg/kg-bw
**Frequency and duration of exposure:** .single dose
**Duration of follow-up:** Twenty four hours after treatment, rats were anesthetized with pentobarbital and necropsied to obtain blood, liver, kidney and brain. Blood was obtained by cardiac puncture and drawn into syringes containing citrate to prevent coagulation.

**Analyses conducted:**

**Extraction of DNA from blood and tissue:** Blood was layered into 15-ml two-chamber tubes containing Histopaque-1077. The Histopaque-1077 kit procedure was used to obtain mononuclear blood cells, which were frozen at -80°C for subsequent DNA extraction Livers and kidney were excised, trimmed, weighed and rinsed in ice-cold 0.15 M NaCl–0.015 M trisodium citrate. Tissues were frozen in liquid nitrogen prior to being stored at -80°C. DNA isolation from liver and kidney were by the procedure of Fiala et al. (1989). DNA was isolated from lymphocytes with Puregene DNA isolation kits.

**ELISA-based measurement in urine of 8OHdG and 8epiPGF:** 8OHdG and 8epiPGF in all urine samples were analyzed by Genox using ELISA-based kits. Standard procedures were followed for both kits.

**HPLC-EC measurement of 8OHdG in urine, tissue and lymphocytes:** DNA extracted from lymphocytes and liver was hydrolyzed by incubating 40–200 mg DNA in 200 ml 20 mM sodium acetate containing 5 ml of 1 U:ml nuclease P1 at 65°C. After 10 min, 10 ml of 1 M Tris–HCl and 5 ml of 1 U:ml of alkaline phosphatase were added and the solution was incubated for 1 h at 37°C. The pH was adjusted to 5.1 with 10 ml of 3 M sodium acetate buffer and the solution was filtered through a 5 k Millipore concentrator tube by centrifuging 5000 x *g* for 10 min. Samples were assayed for 8OHdG using high pressure liquid chromatography and electrochemical detection (HPLC-EC).

**Creatinine:** Creatinine in urine was assayed by the Stanibo Creatinine Procedure No. 400 (Stanibo Laboratory, San Antonio, TX).

**Liver and kidney TBARS:** TBARS were measured in liver and kidney according to Tirmenstein et al. (1995) with the following modification: 1 cm3 tissue pieces were weighed and homogenized in nine parts of 1.15% KCl. The homogenate was centrifuged for 10 min at 1000 x *g*. A 1-ml aliquot was combined with an equal volume of 12% trichloroacetic acid and centrifuged for 10 min at 1000 x *g* to remove precipitated protein. One milliliter of supernatant was added to 1 m thiobarbituric acid reagent (0.6% thiobarbituric acid, 0.01% BHT, 1.0 mm EDTA), and the mixture was heated at 100°C for 20 min prior to extraction of TBARS with 3 ml of 1-butanol.

**Statistical analysis:** All statistical procedures were performed using Statgraphics statistics package. Data were compared using ANOVA. Data were presented as the mean $\pm$S.D. $P<0.05$ was considered statistically significant.

**RESULTS:**

**Morbidity and mortality:** A single exposure to 100 mg/kg-bw of 2-nitropropane was without apparent effect on rats at the time of dosing.

**Body and organ weights:** A single exposure to 2-nitropropane significantly reduced body weights relative to controls and a significant effect on liver/body weight ratio. Kidney and brain weights were not affected.

**Urine volume:** Urine volume was significantly increased for 12 and 24 h in rats dosed with 2-nitropropane.

**Creatinine excretion:** Creatinine excretion decreased significantly in rats dosed with 2-nitropropane.

**8epiPGF excretion:** In 2-nitropropane-exposed rats, urinary 8epiPGF increased significantly during the 24 h after dosing, and was significantly elevated above control values regardless of mode of expression.

**ELISA analysis of 8OHdG excretion:** Urinary excretion of 8OHdG was measured by ELISA in urine excreted 12 h prior to exposure to 2NP, and 12 and 24 h post exposure. Urinary excretion of 8OHdG in 2NP-exposed rats was not significantly increased for the 24 h post exposure. However, when only the 12- to 24-h period was examined, 8OHdG excretion was significantly increased regardless if expressed as total output or per mg creatinine.

**Liver and kidney TBARS:** Treatment with 100 mg/kg-bw of 2-nitropropane, significantly increased TBARS above control value in the liver, but not in the kidney.

**Liver and lymphocyte 8OHdG:** 2-nitropropane significantly increased 8OHdG/dG ratios in liver DNA relative to the control. There were small increases in 8OHdG/dG levels in lymphocytes from rats exposed to 2-nitropropane, but the increase was not statistically significant

**CONCLUSIONS:**

**Assessment of urinary 8OHdG and 8epiPGF are useful biological markers for detecting oxidative damage produced by acute exposure of rats to 2NP and other chemicals.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The purpose of this study was to test the hypothesis that Trichloroethylene (TCE) and perchloroethylene (PERC) increased urinary excretion of oxidative stress biomarkers in experimental animals. Positive results would serve as a foundation for using 8OHdG and 8epiPGF as markers of a biologically-effective dose in studies of workers exposed to TCE and/or PERC. 2-nitroproane was used as positive control.

**REFERENCE:**

Toraason M, Clark J, Dankovic D, Mathias P, Skaggs S, Walker C, Werren D. 1999. Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene. Toxicology 138: 43–53.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, US).

**METHOD:**

**Method/Guideline:** Other.
**Type of study:** Oxidative DNA and RN damage study
**GLP:** Not stated.
**Year:** 1990.
**Species and strain:** Rats, Sprague-Dawley strain.
**No. of animals/sex/dose:** Male and female, numbers not specified.
**Route of exposure:** Intrperitoneal injection
**Vehicle:** 4:1 H20/Emulphor
**Dose/Concentrations:** 1.12 mmol/kg-bw (equal to100 mg/kg-bw)
**Frequency and duration of exposure:** .single dose
**Duration of follow-up:** Rats were killed by decapitation 6 or 18 h following i.p. injection of the test compounds. Livers and kidneys were immediately removed, rinsed with cold 0.15 M NaCl—0.015 M sodium citrate, pH 7.0, quick-frozen in liquid $N_2$, and stored at -70°C until isolation of nucleic acids.
**Analysis conducted:** Lver and kidney DNA were isolated by a modification of the Marmur (1961) procedure; RNA was isolated by the method of Chomczynslri and Sacchi (1987). Nucleic acids were hydrolyzed to nucleosides using nuclease PI and alkaline phosphatase, and stored on ice. Following a modification of the method of Floyd *et al.* (1986), aliquots of the hydrolysates were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC/EC) using a 0.46 x 25 cm Ultrasphere ODS column and a 0.46 x 4.5 cm Ultrasphere ODS guard column. HPLC/EC analyses were performed not later than 6 h after hydrolysis of the nucleic acids.

**Statistical analysis:** Not stated, but data were presented as the mean $\pm$S.D, and $P<0.05$ was considered statistically significant.

**RESULTS:**

2-nitropropane increased 8-hydroxy-2'-deoxyguanosine (8-OH-dG) level in rat liver, 6 and 18 h after administration, which was markedly higher in male rats than in female rats. However, 2-nitropropane did not increase the 8-OH-dG level in the kidney of treated rats.

**CONCLUSIONS:**

**The study result s indicated that significantly less nucleic acid damage occurred in the liver of female SD rats than in the male rats, and that there were virtually no 2-nitropropane induced modifications of kidney nucleic acids detectable by HPLC/EC.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The purpose of this study was to examine the ability of 2-nitropropane or acetoxime to induce base damage in liver and kidney nucleic acid of male and female rats. The study results supported the hypothesis that specific DNA alterations observed are relevant to the hepatocarcinogenicity of 2-nitropropane and acetoxime.

**REFERENCE:**

Guo N, Conaway CC, Hussain NS, Fiala ES. 1990. Sex and organ differences in oxidative DNA and RNA damage due to treatment of Sprague-Dawley rats with acetoxime or 2-nitropropane. Carcinogenesis 11: 1659–1662.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, US).

**METHOD:**

**Method/Guideline:** Comet assay
**Type of study:** DNA damage study.
**GLP:** Not stated.
**Year:** 1997.
**Species and strain:** Rats, Wistar strain.
**No. of animals/sex/dose:** Six male animals/group.
**Route of exposure:** Intraperitoneal injection.
**Vehicle:** Corn oil.
**Dose/Concentrations:** 100 mg/kg-bw.
**Frequency and duration of exposure:** single dose.
**Duration of follow-up:** All rats were killed 24 h after injection. Bone marrow from the two femurs of each rat was flushed out with 3x3 ml of cold phosphate-buffered saline (PBS, 0.1 M, pH 7.4) supplemented with 10 units/ml heparin. The harvested cells were separated and suspended in PBS, and washed twice with 1 ml of PBS. The cells were then resuspended and diluted to about 500 000 cells per ml. Twenty µl of this suspension was used for the comet assay. The remaining cells were stored at –20$^{o}$C for analysis of 8-oxodG in the DNA.

**Analysis conducted:**

**Comet assay:** The comet assay was performed with minor modifications of the technique described by Singh et al. (1988). Eighty-five µl of 1% normal of 1% normal melting agarose in Mg2+ - and Ca2+ - free PBS (0.1 M, pH 7.4) was dissolved and spread onto fully frosted microscope slides and covered with a 18x18 mm coverslip. After 10 min at 4$^{o}$C the coverslip was removed and 10 µl of cell suspension mixed with 85 µl of 1% lower melting agarose at 37 $^{o}$C was pipetted onto the first agaose layer. After 10 min at 4$^{o}$C with a coverslid the slide was immersed into a freshly prepared cold lysis solution at 4$^{o}$C for 1h. The slides were placed horizontally in a tank with electrophoresis buffer for 30 min to unwind DNA. The electrophoresis was carried out at 25 V at room temperature for 25 min and the electric current was adjusted to 300 mA by the buffer level. The slide was then neutralized three times in a solution of 0.4 M tris buffer and stained for 5 min with 85 µl of 0.5 µM YOYO-1 in PBS. Ten minutes after staining, DNA comets were measured with an Olympus image analysis system using an excitation filter of 490 nm and a barrier filter of 530 nm. Cells were identified at 400 x magnification. Brightness and contrast were adjusted to clearly define the comet head and tail borders. One coded slide was evaluated per rat and the observer was unaware of the treatment. Fifty randomly selected cells were measured from each slide. Quantification of the DNA damage was calculated as: Comet tail length (µm) = (maximum head-tail length) – (head diameter).

**Determination of 8-oxodG:** Cells were suspended in 1.8 ml ice-cold TE buffer, and 200 µl 1% dodecyl sulfate sodium salt (SDS) was added. After vortexing for 30 s and incubation in a water bath at 37 °C for 10 min, 200 µl 3 m sodium acetateand 550 µl 5 M sodium perchlorate were added. Chloroform/isoamyl alcohol (24:1) 2 ml was used to purify DNA. After rotation in an extraction bench for 10 min the samples were centrifuged at 3500 rpm for 10 min and 2 volumes of ice-cold 96% ethanol were slowly added to the separated supernatant (non-organic phase). The DNA was allowed to precipitate at -20°C overnight followed by centrifugation at 3000 rpm for 5 min. The DNA precipitate was washed with 70% ethanol, dried with a stream of nitrogen gas, dissolved in 200 µl 20 mM sodium acetate, digested to nucleoside level at 37 °C with 20 µl per sample of Nuclease Pl for 30 min and 20 µl per sample of alkaline phosphatase for another 60 min. The ratio of 8-oxodG to deoxguanosine (dG) was measured using a HPLC system with electrochemical and UV detections.

**Statistical analysis:** 2-nitropropane and vehicle treated rats were compared on a group basis by means of *t*-tests. Probability values less than 0.05 wee considered statistically significant.

**RESULTS:**

The results of this study demonstrated that 2-notropropane inflicts damage to DNA including oxidative modification of guanine in rat bone marrow cells. The 8-oxodG level in the DNA was increased 5-fold by 2-nitropropane.

**CONCLUSIONS:**

**2- nitropropane induced DNA damage in rat bone marrow.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

There was a close correlation between the comet tail length and the 8-oxodG level. The results indicate that 2-nitroppropane inflicts DNA damage in the bone marrow cells and thus could be leukemogenic.

**REFERENCE:**

Deng X-S, Tuo J, Poulsen HE, Loft S. 1997. 2-Nitropropane-induced DNA damage in rat bone marrow. Mutat Res 391: 165–169.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, US).

**METHOD:**

**Method/Guideline:** Comet Assay
**Type of study:** The Comet Assay with multiple mouse organs: comparison of Comet Assay results and carcinogenicity with 208 chemicals selected from the IARC Monographs and U.S. NTP Carcinogenicity Database"
**GLP:** Not stated.

**Year:** 2000.
**Species and strain:** Mice, ddY strain.
**No. of animals/sex/dose:** Four male animals/group.
**Route of exposure:** Intraperitoneal injection.
**Vehicle:** Not stated
**Dose/Concentrations:** 500 mg/kg-bw.
**Frequency and duration of exposure:** single dose.
**Duration of follow-up:** Sample times were 3, 8 and 24 h after treatment.

**Analyses conducted:** No information

**Statistical analysis:** No information

**RESULTS:**

2-nitropropane induced DNA damage in stomach, colon and liver in mice 8 h after treatment. No detectable effects on the kidney, urinary bladder, lung, brain or bone marrow were found.

**CONCLUSIONS:**

**2- nitropropane induced DNA damage in mice stomach, colon and liver.**

**RELIABILITY:**

(4) Not assignable.

**GENERAL REMARKS:**

The purpose of this report was to summarize the *in vivo* genotoxicity in eight organs of the mouse of 208 chemicals selected from International Agency for Research on Cancer (lARC) Groups 1, 2A, 2B, 3, and 4, and from the U.S. National Toxicology Program (NTP) Carcinogenicity Database, and to discuss the utility of the comet assay in genetic toxicology. Because of the nature of this study, no detailed study method and analysis were available.

**REFERENCE:**

Sasaki YF, Sekihashi K, Izumiyama F, Nishidate E, Saga A, Ishida K, Tsuda S. 2000. The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC Monographs and U.S. NTP carcinogenicity database. CRC Crit Rev Toxicol 30: 629–799.

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, US).

**METHOD:**

**Method/Guideline:** Unscheduled DNA synthesis (UDS) and micronucleus test
**Type of study:** Genotoxicity study of 2-nitropropane and its isomer 1-nitropropane
**GLP:** Not stated.
**Year:** 1989.

**Species and strain:** Rats, Sprague-Dawley strain.
**No. of animals/sex/dose:** Five to six animals/group.
**Route of exposure:** Oral administration.
**Vehicle:** Water
**Dose/Concentrations:** 1) UDS test: 0, 25, 50 or 100 mg/kg-bw ; 2) liver micronucleus (LMN) test: 0, 25, 50 or 75 mg/kg-bw; and 3) bone marrow micronucleus (BMMN)test: 0, 50, 100 or 300 mg/kg-bw .
**Frequency and duration of exposure:** single dose.
**Duration of follow-up:** 1) UDS test: 14 to 16 h after dosing, animals were killed with $CO^2$.
Hepatocytes were isolated and cultured according to the procedure described by Ashby *el al.* (1985) except that the perfusion buffers were not gassed with air and no antibiotics were added to these buffers; 2)LMN test: Three days after dosing of the test compounds,
the animals received a single oral dose of 1000 mg/kg 4-acetylaminofluorene
(4-AAF; CAS no. 28322-02-3). Two days later hepatocytes were isolated according to the method developed by Braithwaite and Ashby (1988).; 3) BMMN test: Either 24 or 48 h after dosing, animals were killed by cervical dislocation and bone marrow smears were made according to standard procedures.

**Analysis conducted:**

**UDS test:** The slides were coded and analysed using an image analyser. Either 25 or 50 cells were analysed per slide, and usually three slides per animal.

**LMN test:** The liver micronucleus test was performed according to the method developed by Braithwaite and Ashby as mentioned above. Two methods of slide preparation were used.
***Method A*** (modification of Tates et al. 1980). Immediately after isolation and washing, hepatocytes were fixed directly with methanol/acetic acid (3; 1) by gently pipetting the fixative into the cell suspension. Hepatocytes were spun down (50 *g* for 2 min). The supernatant was removed and the fixation was repeated twice. After this, the hepatocyte suspension was gently run along microscopic slides. The slides were checked under phase contrast (10X magnification) for appropriate cell density and then air dried.
***Method B*** (modification of Braithwale and Ashby). Hepatocytes were allowed to attach for 2 - 3 h on coverslips which were then rinsed with medium, followed by fixation in 85:10:5 methanol/acetic acid/formaldehyde (37%) for 30 min. After this, coverslips were rinsed in distilled water, allowed to dry and mounted on microscope slides. All slides were placed in 5 M hydrochloric acid for 1 h, rinsed in distilled water, left to stand in Schiff s reagent for 20 min followed by distilled water for 10 min. The cytoplasm was then stained with 0.5% light green for 2 min. After air drying, slides were mounted with glass coverslips and coded. For each slide preparation method, a minimum of 2000 hepatocytes per animal were analysed under 1000 X magnification for the presence of micronuclei according to the scoring criteria of Braithwaite and Ashby. On the slides made by method A, the number of mitoses was determined in at least 2000 hepatocytes per animal.

All slides were placed in 5 M hydrochloric acid for 1 h, rinsed in distilled water, left to stand in Schiff s reagent for 20 min followed by distilled water for 10 min. The cytoplasm was then stained with 0.5% light green for 2 min. After air drying, slides were mounted with glass coverslips and coded.

For each slide preparation method, a minimum of 2000 hepatocytes per animal were analysed under 1000 X magnification for the presence of micronuclei according to the scoring criteria of Braithwaite and Ashby. On the slides made by method A, the number of mitoses was determined in at least 2000 hepatocytes per animal.

**BMMN test:** Either 24 or 48 h after dosing, animals were killed by cervical dislocation and bone marrow smears were made according to standard procedures. To avoid the staining of mast cell granules, slides were stained with haematoxylin-eosin. Slides were coded prior to analysis. Two thousand polychromatic erythrocytes (PE) per slide were analysed for the presence of micronuclei, and 500 erythrocytes per slide were scored to determine the proportion of PE among all erythrocytes.

**Statistical analysis:** 1) UDS test: no information; 2) LMN test: data were checked for normal distribution and then compared by either Student's t-test or by analysis of variance; 3) BMMN test: Statistical analysis was performed according to Amphlett and Delow (1984).

**RESULTS:**

**UDS test:** 2-nitropropane induced DNA repair in a dose-dependent manner between 50 -100 mg/kg-bw, but it did not induce UDS at 25 mg/kg-bw.

**LMN test:** 2-nitropropane induced micronuclei in the liver. At the two low doses (25 and 50 mg/kg-bw), the effect was statistically significant. In the high-dose group, the effect was induced , but not significant.

**BMMN test:** 2-nitropropane slightly increased micronucleus frequency in bone marrow at the highest dose (300 mg/kg-bw), but not statistically significant. Negative results were obtained in all lower dose groups.

**CONCLUSIONS:**

**2- nitropropane induced UDS and micronuclei in the rat liver. In bone marrow, however, it only slightly increased micronuleus frequency at the highest dose level.**

**RELIABILITY:**

(2) Valid with restrictions.

**GENERAL REMARKS:**

The purpose of this study was to investigate the organ- or endpoint- specificity of 2-nitropropane.

**REFERENCE:**

George E, Burlinson B, Gatehouse D. 1989. Genotoxicity of 2-nitropropane in the rat. Carcinogenesis 10: 2329–2334

**CHEMICAL:**

**Name (CAS No.):** 2-Nitropropane (CAS RN 79-46-9)
**Purity:** Not stated (obtained from Wako)

**METHOD:**

**Method/Guideline:** Micronucleus assay
**Type of study:** Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (group 1, 2A and 2B)
**GLP:** Not stated.
**Year:** 1997.
**Species and strain:** Mice, CD-1 strain.
**No. of animals/sex/dose:** Five male animals/group.
**Route of exposure:** Intraperitoneal injection.
**Vehicle:** Olive oil
**Dose/Concentrations:** 0, 125, 250 or 500 mg/kg bw.
**Frequency and duration of exposure:** two times for each dose, intervals between them were 24 h.
**Duration of follow-up:** Micronuclei were scored at 0, 24, 48 and 72 h after final treatment.
**Analyses conducted:** No detailed information was available. However, the organizing committee proposed minimum requirements for the micronucleus assay which included that both bone marrow polychromatic erythrocytes and peripheral blood reticulocytes were used for analysis; and that

micronucleated polychromatic erythrocytes and peripheral blood reticulocyte frequencies were based on the observation of at least 1000 polychromatic erythrocytes or reticulocytes per animal.
**Statistical analysis:** Data were analyzed by the modified 3-step procedure of Hayashi et al. (1989b, 1994a) and Adler et al. (1996), and were presented as the mean ±S.D, and *p*- values.

**RESULTS:**

2-nitropropane did not induce micronucleated polychromatic erythrocytes or micronucleated reticulocytes in treated mice.

**CONCLUSIONS:**

**2- nitropropane did not induce micronuclei in mice bone marrow or peripheral blood.**

**RELIABILITY:**

(4) Not assignable.

**GENERAL REMARKS:**

The purpose of this report was to assess the correlation between micronucleus induction and human carcinogenicity. The results of the study demonstrated that the mouse erythrocyte micronucleus assay detected IARC human carcinogens according to the degree of evidence for carcinogenicity. However, the assay showed different sensitivity to different chemical classes.

**REFERENCE:**

Morita T, Asano N, Awogi T, Sasaki YF, Sato S-I, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M. 1997. Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS.MMS. Mutat Res 389: 3–122.

| | Gene Mutation | DNA damage | Chromosomal aberration | Micronucleus | Sister chromatid exchange (SCE) |
|---|---|---|---|---|---|
| <u>*In vitro*</u> Genotoxicity studies with 2-Nitropropane (CAS RN 79-46-9) | + (*Salmonella typhimurium* TA98, TA100 and TA102 with or without metabolic activation) - (*Salmonella typhimurium* TA1535 and TA1537) (IARC 1999)<br><br>+ (Chinese hamster lung V79 cells, *hprt* locus and rat hepatoma H4IIEC3/$G^-$ cells) (Roscher et al. 1990) | + (human, rat and mouse primary hepatocytes) (Davies et al. 1993)<br><br>+ (rat primary hepatocytes) (Kohl et al. 1994; Fiala et al. 1995) | + (human lymphocytes with metabolic activation) (Bauchinger et al. 1987; Goegglemann et al. 1988) | + (H4IIEC3/$G^-$ cell line, 2sFou rat hepatoma cell line and $C_2$Rev7 rat hepatoma cell line without metabolic activation) (Roscher et al. 1990)<br><br>- (Chinese hamster lung V79 cells without metabolic activation) (Roscher et al. 1990) | + (human lymphocytes with metabolic activation (Bauchinger et al. 1987; Göggelmann et al. 1988)<br><br>- (Chinese hamster ovary cells with or without metabolic activation (Galloway et al. 1987) |

**+ positive result**
**- negative result**

**REFERENCES:**


Bauchinger M, Kulka U, Schmid E. 1987. Analysis of cytogenetic effect in human lymphocytes induced by metabolically activated 2-nitropropane. Mutat Res 190: 217–219. [cited in IARC 1999].

Davies JE, Mynett K, Gescher A, Chipman JK. 1993. DNA modification and repair by 2-nitropropane is extensive in hepatocytes of rats compared to those of humans and mice. Mutat Res 287: 157–164. [cited in IARC 1999].

Fiala ES, Sodum RS, Hussain NS, Rivenson A, Dolan L. 1995. Secondary nitroalkanes: induction of DNA repair in rat hepatocytes, activation by aryl sulfotransferase and hepatocarcinogenicity of 2-nitrobutane and 3-nitropentane in male F344 rats. Toxicology 99: 89–97.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin GH, Resnick MA, Anderson G, Zeiger E. 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ Mol Mutagen 10(Suppl 10): 1–175. [cited in IARC 1999].

Göggelmann W, Bauchinger M, Kulka U, Schmid E. 1988. Genotoxicity of 2-nitropropane and 1-nitropropane in *Salmonella typhimurium* and human lymphocytes. Mutagenesis 3: 137–140. [cited in IARC 1999].

[IARC] International Agency for Research on Cancer. 1999. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 71, part 3. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. Lyon, France.

Kohl C, Mynett K, Davies JE, Gescher A, Chipman JK. 1994. Propane 2-nitronate is the major genotoxic form of 2-nitropropane. Mutat Res 321: 65–72. [cited in IARC 1999].

Roscher E, Ziegler-Skylakakis K, Andrae U. 1990. Involvement of different pathways in the genotoxicity of nitropropanes in cultured mammalian cells. Mutagenesis 5: 375–380. [cited in WHO 1992].

[WHO] World Health Organization. 1992. 2-Nitropropane [Internet]. Geneva (CH): World Health Organization. Environmental Health Criteria 138. Available from: http://www.inchem.org/documents/ehc/ehc/ehc138.htm