

Review of Genotoxicity of Acrylamide

Summary Of Mutagenicity Data Summarised From European Chemical Bureau Eu Risk Assessment Report (1st Priority List Volume 24, 2002.)

1. A brief overview of the mutagenicity data reviewed in the EU risk assessment review drafted by HSE and agreed by an EU Technical Working Group. Published literature up to 1995 were included.

In vitro Mutagenicity

Bacterial tests

Reference	Method	Result/Comments
HSE EU Risk Assessment 2002 (A1) Bull et al 1984, Godek et al 1982a, Hashimoto and Tanii 1985, Jung et al 1992, Knaap et al 1988, Lijinsky and Andrews 1980, Muller et al 1993, Tsuda et al 1993, Zeiger et al 1987	Range of well conducted published and unpublished liquid preincubation and plate incorporation tests in <i>Salmonella typhimurium</i> TA98, TA100, TA102, TA1535, TA1537, TA1538 in presence and absence of exogenous metabolic activation at 100-50,000 µg/ml	Negative results in all assays.
HSE EU Risk Assessment 2002 (A2) Bull et al 1984, Godek et al 1982a, Hashimoto and Tanii 1985, Jung et al 1992, Knaap et al 1988, Lijinsky and Andrews 1980, Muller et al 1993, Tsuda et al 1993, Zeiger et al 1987	Range of well conducted tests using <i>Escherichia coli</i> WP ^u uvrA- in presence and absence of exogenous metabolic activation at 100-50,000 µg/ml	Negative result in all assays.
HSE EU Risk Assessment 2002 (A2) Knaap et al 1988	Fluctuation test for streptomycin resistance in <i>Klebsiella pneumoniae</i> at 100-10,000 µg/ml.	Negative. Mutation frequency not significantly altered.
HSE EU Risk Assessment 2002 (A2) Vasavada and Padayatty 1981	Bacterial transfection assay using <i>Escherichia coli</i> CR63 cells at up to 10 µg.	Positive. Concentration related increase in inhibition of transfection (reported to indicate mutagenic activity). Significance of finding unclear.

2. Overall negative results have been reported in standard bacterial mutagenicity tests both in presence and absence of exogenous metabolic activation.

In vitro cytogenetics assays in Mammalian cells.

HSE EU Risk Assessment 2002 (A1) Knaap et al 1988	Well conducted assay using V79 Chinese hamster cells at 0-3,000µg/ml in presence and absence of exogenous metabolic activation (Test material >98%)	Positive. Dose related increase in chromosomal aberrations reported in both presence and absence of exogenous metabolic activation. No data on cytotoxicity reported.
--	---	---

HSE EU Risk Assessment 2002 (A2) Tsuda et al 1993	Cytogenetics assay using V79H3 Chinese Hamster cells exposed to 0-5 mM (0-355 µg/ml) acrylamide (>99%) in absence of exogenous metabolic activation. The exposure period was 24 h followed by fixation for further 20 or 40 hours.	Positive. Dose related increase in metaphases with chromosomal aberrations and polyploidy was reported. Frequency exceeded positive control MNING. No information on cytotoxicity report.
--	--	---

3. The EU risk assessment review concludes that acrylamide is a direct acting clastogen in mammalian cells in vitro.

In vitro Mammalian cell gene mutation assays

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Tsuda et al 1993	V79H3 cells (HPRT locus) exposed in absence of exogenous metabolic activation to 0-7 mM (0-500µg/ml) acrylamide for 24 hours.	Negative. There was no evidence for a treatment related increase in mutation frequency. Survival at 213 µg/ml survival was 59% and at 355 µg/ml 21% or less.
HSE EU Risk Assessment 2002 (A1) Godek et al 1982 Godek et al 1984	CHO cells exposed to 37.5-900 µg/ml acrylamide (unknown purity) with and without S9 for 5 hours.	Negative. A slight dose related increase in mutant frequency (50% above solvent control value) was observed at 300 µg/ml and above in presence of exogenous metabolic activation and at all exposure levels without exogenous metabolic activation. In a follow up study by the same group, negative results were obtained at up to 1500 µg/ml. It is noted that cell survival was reported to be about 70%. Positive control substances gave expected responses (EMS/DMN). (Godek 1982b and 1984)
HSE EU Risk Assessment 2002 (A1) Knaap et al 1988	Exposure of mouse lymphoma cells (L5178Y TK+/-) to 0-7,500 µg/ml with and without exogenous metabolic activation (primary rat liver or Syrian hamster ovary cells) for 2h, 4h or 24h.	A slight dose-related increase in mutation frequency was noted for each exposure period both in presence and absence of exogenous metabolic activation at dose levels associated with low survival (usually less than 30%). (Knapp 1988)
HSE EU Risk Assessment 2002 (A1) Moore et al 1987	Exposure of mouse lymphoma cells (L5178Y TK+/-) to 0-850 µg/ml (>99% purity). For 4 hours without metabolic activation.	An increase in mutation frequency was noted at 500 and above (40% survival at 500 µg/ml, 10% survival at 850 µg/ml). There was a dose related increase in the frequency of small colonies and at 750 µg/ml and above mutant colonies were mainly considered to be small size. (A separate assay by these investigators reported chromosome breaks and rearrangements in this cell line.) (Moore 1987)

4. The EU risk assessment review concludes that acrylamide is a direct acting mutagen in the available assays probably causing clastogenic effects.

In-vitro DNA synthesis and repair

Reference	Methods	Results/Comments
HSE EU Risk Assessment 2002 (A1) Naismith and Matthews 1982	UDS was measured in freshly isolated rat hepatocytes following incubation with acrylamide at 0-100 mg/ml for 18-20 hours.	Positive. NNG counts were significantly elevated above controls and achieved levels close to the positive control 2-AAF. There was no clear dose response. Viability of harvested cells was 88%. Results confirmed in a repeat experiment.
HSE EU Risk Assessment 2002 (A1) Miller and McQueen 1986	UDS was measured in rat hepatocytes incubated with acrylamide at 0-3.55 mg/ml 0-50 mM for 18 hours.	Negative. No evidence for UDS reported (using ≥ 5 NNG greater than controls as criteria for positive). No clear dose response reported. Positive control 2AF gave expected response. Cytotoxicity was observed at the highest concentration.
HSE EU Risk Assessment 2002 (A1) Butterworth et al 1992	UDS was measured in rat hepatocytes incubated with acrylamide at 0-710 μ g/ml (0-10 mM)	Negative. No significant increase in number of NNG compared to negative control. Toxicity (morphological assessment) was noted at the highest concentration. Positive results seen with DMN control.

5. The EU risk assessment review considers that the available studies are inconsistent and it is difficult to draw a definite conclusion.

Other in vitro data reported in EU risk assessment review.

6. Slight, reportedly statistically significant increases in SCEs were reported in V79 H3 Chinese hamster cells (0-3 mM, 0-213 μ g/ml, >99% purity) without metabolic activation and in V79 cells (0-3,000 μ g/ml with and without exogenous metabolic activation). A number of positive results have been reported in cell transformation assays both in presence and absence of exogenous metabolic activation. These tests are generally not used by COM in deriving conclusions with regard to in vitro genotoxic activity.

Overall conclusion *in vitro* mutagenicity (based on EU risk assessment report)

7. The EU risk assessment report presents a conclusion that acrylamide is a direct acting clastogen in mammalian cells producing chromosomal aberrations and polyploidy. Supporting evidence for clastogenicity came from the mammalian cell gene mutation assays. The possibility of activity via exogenous metabolic activation cannot be excluded from the information available in the EU review.

In vivo Mutagenicity tests

Somatic cells

Cytogenetics

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Cihak and Vontorkova 1988	Groups of 5 male mice given i.p. 0 or 100 mg/kg bw acrylamide (aqueous) with 50 bone marrow cells evaluated/dose level at 6, 18, 24 and 48 h post dose.	Statistically significant increase in metaphases with chromosome or chromatid breaks (3-11% cf 1% in controls) at 18, 24, and 48 h post dose. No positive control used.
HSE EU Risk Assessment 2002 (A1) Adler et al 1988	Groups of 5 male and 5 female were given single i.p. doses of 0 or 100 mg/kg bw (in saline). Samples of bone marrow were taken at 12, 18, 24, 30, and 36 h for analysis of chromosome aberrations. A subsequent dose response study was undertaken using 0, 50, 100 and 150 mg/kg bw with an 18 h sampling time	Statistically significant increases in metaphases with chromosome and chromatid breaks were reported (2.6-4.4% cf 0.7% in controls) with a maximal response at 18h. In dose response study significant increases in the frequency of aberrant cells was seen at all doses (2.1-4.1% excluding gaps compared to 0.3% in

	point.	negative control and 3.6% in positive (cisplatin) control. Mitotic index was reduced by up to 27% compared to controls.
HSE EU Risk Assessment 2002 (A1) Shiraishi 1978	Groups of 5 male mice were given 500 ppm acrylamide in diet (estimated to be approximately 60 mg/kg bw/day) for 1,2 or 3 weeks. Animals were sacrificed at the end of the dietary administration and 100 bone marrow cells scored for chromosome breaks. A further group of animals was given 0 or 100 mg/kg bw i.p. (vehicle not given) and bone marrow cells assessed for chromosomal damage at ½, 1, 11 or 12 days post administration.	A slight increase in chromosome damage was reported following dietary administration. The authors considered this to be a negative response. The EU risk assessment review suggests there is uncertainty regarding this conclusion. It is noted that there were no statistical analyses presented. Following i.p. administration there was a slight increase in chromosome breaks (2.7% control, 3.5-7% in treated) and an increase in aneuploidy or polyploidy was also reported (3.5% in controls and 5-10.5% in treated animals). There were no data on different sampling times available. No data for positive control experiments were reported. (An increase in SCEs/cell was reported in the dietary experiment).
HSE EU Risk Assessment 2002 (A1) Backer et al 1989	Groups of 4 male mice were given a single i.p dose of acrylamide (vehicle not given) at 0, 50, 100, or 125 mg/kg bw. Mitoses from 100 splenocytes were examined 24 hours post administration.	A non-statistically significant increase in chromatid aberrations (5% at 125 mg/kg cf 2% in controls). A dose related increase in SCEs was reported. Positive controls received cyclophosphamide.

8. Overall acrylamide produces chromosome aberrations in bone marrow with limited evidence regarding splenocytes. No studies for other somatic cells were available in the EU risk assessment review.

Micronucleus assays

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Adler et al 1988	Groups of 5 male and 5 female were given single i.p. doses of 0 or 100 mg/kg bw (in saline). Samples of bone marrow were taken at 12, 18, 24, 30, and 36 h for analysis of chromosome aberrations. A subsequent dose response study was undertaken using 0, 50, 75 and 125 mg/kg bw with an 24 h sampling time point.	Statistically significant increases in MN reported at 18, 24 (max 0.66% cf 0.13% in controls) and 30 h. A clear increase in MN was reported at all dose levels in the dose-response study with a frequency of 0.9% at 125 mg/kg bw (cf 1% using cisplatin positive control).
HSE EU Risk Assessment 2002 (A1) (Backer et al 1989, Cao et al 1993, Cihak and Vontorkova 1988, Cihak and Vontorkova 1990, Knaap et al 1988, Russo et al 1994)	Bone marrow, spleen or peripheral blood assays using single or repeated doses i.p up to 150 mg/kg bw (aqueous) and sampling times of between 6-72h.	Positive results in all assays, with peak effects generally at 24 h.
HSE EU Risk Assessment 2002 (A1) (Sorg et al 1982a)	Bone marrow MN assay using male and female mice given 75 mg/kg bw as an oral aqueous dose (singly or repeated (2x)). Sampling at 30, 48	Negative result. Sampling times earlier than 30 h not used. Positive control triethylenemelamine given i.p.

and 72 h.

9. Acylamide induces MN in bone marrow and spleen.

Liver UDS

Reference	Method	Result/Comments
HSE EU Risk Assessment 2002 (A1) (Butterworth et al 1992)	Groups of rats were given single or 5x i.p doses of 0, 30, 100 mg/kg bw acrylamide. Animals were sacrificed 2 or 12 hours after a single dose or 4h after the last of the repeated doses. Hepatocytes were isolated and incubated with tritiated thymidine for 4h.	No increases in NNG reported. Positive control DMN produced clear positive response.

10. Acylamide did not induce liver UDS in the one available study.

Other somatic cell assays.

11. A positive result was reported in a mammalian spot tests where groups of 31-93 pregnant female mice received a single or 3 daily i.p. doses of 0, 50 or 75 mg/kg bw aqueous acrylamide on day 12 or days 10,11, and 12 of gestation. Approximately 220-300 offspring/dose level were examined. Single or repeated dosing of 50 mg/kg bw was positive. A single dose of 75 mg/kg bw was positive but repeated doses of 75 mg/kg bw were embryotoxic and cytotoxic. A positive result was noted in a LacZ transgenic mutation assay ($62-89 \times 10^6$ cf $15-26 \times 10^6$ in controls) where groups of mice were given 5 daily i.p. dose of 50 mg/kg bw acrylamide and bone marrow samples examined for mutation frequency on days 3,7, and 10 post dose. (Hoorn et al 1993 and Mhyr 1991) The EU risk assessment report considered the full significance of the unvalidated assay reviewed was unclear.

12. A mixture of positive and negative results have been reported for tests using Drosophila.

Overall conclusion in vivo somatic cell mutagenicity studies (based on EU risk assessment report)

13. The EU risk assessment review presents a conclusion that acrylamide is clearly mutagenic in vivo particularly in the bone marrow. The pattern suggested a clastogenic effect or interference with mitosis rather than gene mutation activity. However it is noted that gene mutation activity cannot be excluded on the basis of the transgenic studies in mice reported in the review.

In vivo Germ cell assays

Cytogenetics

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) (Pacchierotti et al 1994)	Groups of 5-16 male mice were dose i.p. with 0, 75, 125 mg/kg and mated with untreated females at 7 or 28 days (125 mg/kg bw) later. A separate group received 5x 50 mg/kg/d with mating 7 days post dosing. Flow cytometry undertaken for cells taken with testicular preparations at 3 and 35 days after treatment (with up to 150 mg/kg bw).	A dose-related increase in chromosomal aberrations was reported in one-cell zygotes at day 7 with a lesser effect at day 28. For repeated dosing 85% of zygotes contained aberrations. For testicular cells there was a marked reduction in tetraploid cells 3 days after dosing. At 35 days there was a dose-related increase elongated diploid spermatids (suggesting impaired segregation). A decrease in diploid spermatids was noted 3d after single dosing but not after repeated dosing which was suggested to indicate an effect on spermatocytes during meiosis from initial dosing.
HSE EU Risk	Groups of 5 male mice were given	An increase in incidence of spermatogonia with

Assessment 2002 (A1) Shiraishi 1978	500 ppm acrylamide in diet (estimated to be approximately 60 mg/kg bw/day) for 1,2 or 3 weeks. Animals were sacrificed at the end of the dietary administration and 100 spermatogonia scored for chromosome breaks. A further group of animals was given 0 or 100 mg/kg bw i.p. (vehicle not given) and spermatogonia assessed for chromosomal damage at ½, 1, 11 or 12 days post administration. In addition 50-100 spermatocytes in diakinesis-metaphase I were examined	aneuploidy, chromosome breaks and SCEs was reported with both exposure regimes. In primary spermatocytes there was a marked increase in sex chromosome and autosomal univalents, fragments and rearrangements following both dosing regimes.
HSE EU Risk Assessment 2002 (A1) Backer et al 1989	Groups of 4 male mice were given a single i.p. dose of acrylamide (vehicle not given) at 0, 50, 100, or 125 mg/kg bw. Mitoses from spermatogonia and spermatocytes were examined 24 hours post administration.	There were no significant changes in the number of chromosome/chromatid aberrations or hyperploidy compared to negative controls. Study limited by one sampling time point.
HSE EU Risk Assessment 2002 (A1) Smith et al 1986	Cytogenetic examination undertaken on rat spermatocytes as part of a dominant lethal study taken from 10-11 males exposed to 0,1.5,3 or 6 mg/kg/d acrylamide in drinking water for 80 days and after a 12 week recovery period.	No statistically significant increase in structural aberrations on completion of 80 days. Slight increase in reciprocal translocations noted in treated animals (0,1,1,2 in each of the groups respectively). No other details reported. Noted that a significant increase in pre-implantation loss occurred.

Germ cell micronucleus assays

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Lahdetie et al 1994	Groups of 5 male rats received single i.p. doses of 0, 50, 1200 mg/kg bw. Animals sacrificed 1,3,18,19 post dosing. 2000 spermatids/animal examined. In a separate study groups of animals were dosed daily over four days at 50 mg/kg bw/d and spermatids analysed as before.	Day 1 = diplotene-diakinesis stage, Day 3= late pachytene, Day 18/19 = spermatogonia. A statistically significant increase in micronucleated spermatids was observed on day 18 following repeated exposure to 50 mg/kg bw
HSE EU Risk Assessment 2002 (A1) Xiao and Tates 1994	Groups of 4-5 male rats were dosed 0, 50, 100 mg/kg bw i.p. or 4 daily i.p.doses of 50 mg/kg bw. Spermatocytes analysed at days 1,3,15,18,19, 20 post dosing.	Statistically significant increases in micronucleus formation were reported on days 18-20 post administration at all dose levels (maximum response at day 19). The effect was more marked for 4x 50 mg/kg bw. Slight but statistically non significant increases were also documented at days 1-3 post administration.
HSE EU Risk Assessment 2002 (A1) Russo et al 1994	No details	Increase in Golgi-phase and Cap-phase spermatids (post meiotic) reported in mice. In addition increased SCE in spermatogonia.

DNA Synthesis and repair

Reference	Method	Results/Comments
-----------	--------	------------------

HSE EU Risk Assessment 2002 (A1) Sega 1990	UDS analysis undertaken for sperm from caudal epididymides. Groups of 4-6 mice were given single i.p. doses of 0, 8, 16, 31, 63, or 125 mg/kg bw. Tritiated thymidine injected into the testes 0-48 h post acrylamide dosing. Epididymid recovered 16 days post dosing. In addition Groups of mice were dosed with 0 or 125 mg/kg bw and tritiated thymidine injected into testes 6h later and sperm from caudal epididymis recovered at 2-3 day intervals up to 30 days post dosing for UDS. DNA was extracted from liver and testes samples 1-24h post administration and analysed for radioactivity.	An increase in UDS was reported with a maximum 6 h post injection of tritiated thymidine equating to early spermatid stage at the time of acrylamide exposure. NOEL? In the second experiment no response was reported during the first 10 days post acrylamide dosing, but from days 12-27 a positive UDS response was seen. In the third experiment DNA alkylation reached a maximum 4-6 post administration into testes, and 1-2 h in the liver, with levels 10x lower in testes compared to liver.
HSE EU Risk Assessment 2002 (A1) Butterworth et al 1992	Groups of F344 rats were dosed with one of five daily doses i.p. of 0, 30, or 100 mg/kg bw. For single dosing animals sacrificed 2 or 12 hours post dose and for repeated dosing 4 hours after the last dose.	Following repeated dosing at 30 mg/kg bw there was a statistically significant increase in NNG. (5.4 and 5.6 cf 0 in control ? results) Appropriate response was reported for positive controls (MMS and cyclophosphamide, 4.9 and 6.5 respectively) A slight but not statistically significant increase was reported following single doses. Overall a positive UDS response was reported.

Dominant Lethal assays

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Gutierrez-Espeleta et al 1992	Groups of 24-30 male mice received 0, 25, 50, 75, 100 or 125 mg/kg bw in aqueous methanol (70%) by dermal administration for 5 consecutive days. Males were mated from day 7-10 after last exposure.	There was a slight but treatment-related decrease in the mean number of implantations per pregnant female. There was a dose related increase in the percentage of dead implants and number of pregnant females with one or more dead implant compared to controls. There was a decrease in the number of liver embryos. A dominant lethal effect was reported following 5 dermal administrations of 25 mg/kg bw/day and above.
HSE EU Risk Assessment 2002 (A1) Shelby et al 1986	Male mice were given a single i.p dose of 125 mg/kg bw or 5 daily injections of 50 mg/kg bw/d.	Dominant lethality reported by increased frequency of dead implants particularly over days 4-12 post dosing. Results considered to be suggestive of an effect on late spermatids and early spermatozoa.
HSE EU Risk Assessment 2002 (A1) Bishop et al 1991	Male mice received 0, 0.7, 2.1, 6 mg/kg/d in drinking water for 140 days. Males mated 16 days later.	A significantly higher percentage of resorptions was found at 6 mg/kg bw/d. Noted that study reported only in abstract with limited details available.
HSE EU Risk Assessment 2002 (A1)	Review of other studies in mice	Positive results for dominant lethality in mice reported in heritable translocations tests, combined DL/2gen reproduction study, and in a cross over breeding study.
HSE EU Risk Assessment 2002 (A1) Smith et al 1986	Groups of 10-11 male rats exposed to 0, 1.5, 3, 6 mg/kg bw in drinking water for 80 days. Mated with untreated females 72 hours post dosing.	Significant preimplantation losses at the highest dose. Post implantation losses increased at 3 and 6 mg/kg bw/d.
HSE EU Risk Assessment 2002 (A1)	Review of other studies	Positive results for dominant lethality obtained in reproductions studies (100 mg/kg bw/d for 5 days by gavage) and in combined two generation DL study (5 mg/kg bw/d in drinking water for 10 weeks).

Heritable Translocation Assays

Reference	Method	Result/Comments
HSE EU Risk Assessment 2002 (A1) Shelby et al 1987	Groups of 120 male mice were dosed i.p. with 0, 40, 50 mg/kg bw /d for 5d and then mated 7-10 post final dose with untreated females.	These dose levels resulted in evidence for dominant lethality, male sterility. Cytogenetic examination of spermatocytes from a group of 10 males (presumably per dose level) found evidence for translocations in all animals. It was reported that 31- 85% of females mated with semi sterile males carried dead implants compared to 0-9% of females mated with apparently non-sterile males
HSE EU Risk Assessment 2002 (A1) Adler et al 1994	Male mice were dosed i.p. with 0, 50, 100 mg/kg bw/ of for 5 days with 50 mg/kg bw/d.	Dominant lethal effects noted at highest dose. An exposure related increase in heritable translocations was reported.

Specific locus assays

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Ehling and Neuhauser-Klaus 1992	Groups of male mice were dosed i.p. with 0, 100 or 125 mg/kg aqueous acrylamide and serially mated with untreated females (homozygous for a number of key physical features).	A high frequency of specific-locus mutations was noted for males treated with females 5-8 days and 9-12 days after injection indicating an effect on late spermatid and spermatozoal stages.
HSE EU Risk Assessment 2002 (A1) Russell et al 1991	Male mice were given 5 doses of 50 mg/kg bw/d i.p.	Increased specific locus mutations were observed at days 8-14 and 15-21 post dosing suggesting effects on spermatid and spermatozoal stages.

Studies in transgenic mice

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Murti et al 1994	As part of validation study, male mice were dosed i.p. with 0 or 50 mg/kg bw/d for 5 days. Testicular preparations examined for <i>LacZ</i> mutations 21-23 days later.	No clear increase in mutations noted. Study investigators considered histological evidence for large cells to be interkinetic delay during meiosis.

Other Germ cell assays

Reference	Method	Results/Comments
HSE EU Risk Assessment 2002 (A1) Sega and Generoso 1990	Groups of male mice dosed i.p. with 0 or 100 mg/kg bw acrylamide following testicular injection of H ³ -thymidine and mature spermatozoa examined up to 21d post dose.	A significant increase in single strand breaks was observed with the greatest elution rate being in the 2 nd week post dosing. (effects were reported for early-mid late spermatids and pachytene spermatocytes.

Other relevant data

14. Evidence for DNA, RNA, and protein binding was reported in a wide range of tissues from mice dosed orally or dermally with ¹⁴C-acrylamide (Carlson and Weaver 1985, Carlson et al 1986). In a separate study groups of male mice were given a single i.p. dose of acrylamide and DNA (measured by purification and radioactivity quantification) and protein binding in epididymis and vas deferentia examined at time points up to 23 days post dose. DNA binding was documented in sperm heads (at approximately 0.5% of the total binding). The predominant DNA binding in this study was found in the liver.

Overall conclusion In vivo studies in germ cells

15. The EU risk assessment review presents a conclusion that acrylamide is clearly mutagenic in vivo in germ cells from studies using a number of different end points (including chromosome aberrations, micronucleus formation, UDS, dominant lethal assays, heritable translocation assays and specific locus assays).

Overall conclusion mutagenicity

16. The EU risk assessment review presents a conclusion that acrylamide is an *in vitro* and *in vivo* somatic cell and germ cell mutagen and has been shown to induce heritable mutations. The EU risk assessment report notes that acrylamide is not mutagenic in bacteria *in vitro*.