Indigotine lake

Toxicological Data on the Unburnt Ingredient

[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]

In vivo

Species	Test conditions	Endpoint	Results	Reference
No relevant data were identified on Pigment Blue 63 (CAS no. 16521-38-3). The data below relate to indigotine (CAS no. 860-22-0).				
Mouse (5 per group)	Animals fed 500 or 1000 ppm in the diet (approx. 75 or 150 mg/kg bw/day) for 90 days and examined for chromosome aberrations in the bone marrow.	Chromosome damage	+ve	Das & Giri, 1988
Mouse (Swiss; group of 10 males)	Animals given 2 mg/kg bw/day by gavage for 30 days and examined for chromosome aberrations in the bone marrow.	Chromosome damage	+ve	Giri et al. 1986
Mouse (Swiss; 5 males per group)	Animals given a single intraperitoneal injection of 25 - 100 mg/kg bw and the bone marrow	Chromosome effects	+ve	Giri & Mukherjee, 1990

	cells examined for sister chromatid exchanges 24 hours after dosing.			
Mouse	Animals possibly given oral doses of 50 mg/kg bw/day for an unknown number of days and examined for chromosome aberrations in the bone marrow. No further details could be extracted from the [presumably] Russian publication which contains a very brief and unclear English abstract.	Chromosome damage	-ve (?) The investigators' concluded that "the doses of these dyes applied in food industry are fairly safe".	Karplyuk et al. 1984
Mouse (BALB/C; 17 per group)	Animals given 100 mg/kg bw by gavage and the bone marrow examined for micronuclei. No further	Chromosome damage	-ve	Tarján & Kürti, 1982a & b

	details available from this abstract.			
Mouse, males (C57Bl/6)	Animals given oral doses of 1.4 or 14 mg/kg bw/day for 5 days and chromosome damage [presumably aberrations in appropriate cells] measured. No further details given in the brief English abstract of the Russian publication.	Chromosome damage	-ve	Durnev et al. 1995
Mouse (4 per group)	Comet assay. Mice given 2 g/kg body weight orally and sacrificed 3 and 34-hours after exposure. DNA damage examined in the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow.	DNA damage	-ve A good quality study.	Sasaki et al. 2002
Mouse	Dominant	Germ cell mutation	-ve	Karplyuk et al.

(CBAxC57BL/6; 10 males)	lethal assay. Animals given 50 mg/kg bw/day orally for 5 months and then mated every week for 8 wk with untreated females (three per male per week). Pre- and post-implantation mortality assessed. No further details available from the very brief and unclear English abstract of this [presumably] Russian publication.		The investigators concluded that "the doses of these dyes applied in food industry are fairly safe".	1984
Rat (Sprague-Dawley; 2 males)	Hepatocyte DNA repair assay. Animals given a single dose of 50 mg/kg bw by stomach tube and evidence of DNA repair in the liver cells examined 2 and 15 hr after dosing.	DNA damage	-ve	Kornbrust & Barfknecht, 1985

In vitro

Test system	Test conditions	Endpoint	Activation status	Results	Reference
No relevant data were identified on Pigment Blue 63 (CAS no. 16521-38-3). The data below relate to indigotine (CAS no. 860-22-0).					
Human peripheral blood lymphocytes	Cells treated with up to 0.1 mg/ml and examined for chromosome aberrations.	Chromosome damage	Without	-ve A limited assay, tested in absence of activation.	Zhurkov, 1975
Chinese hamster lung cells	Cells treated with, probably, up to 8 mg/ml and examined for chromosome aberrations. No further details available in the citing source.	Chromosome damage	With and without S9	+ve with S9	Ishidate, 1987
Chinese hamster lung fibroblast cells	Cells treated with up to at least 12 mg/ml and examined for chromosome aberrations and polyploidy.	Chromosome damage	Without	+ve for polyploidy -ve for chromosome aberrations A limited study, tested in absence of activation.	Ishidate et al. 1980 & 1984
Hamster lung cells	No details available in the citing sources.	Chromosome damage	Without	-ve	Kawachi et al. 1980 & 1981

Chinese hamster ovary cells	Cells treated for 5 hr with up to 20 µM and examined for chromosome aberrations. Apparently not a food grade additive.	Chromosome damage	Without	Weak +ve	Au & Hsu, 1979
Mouse lymphoma cells	Cells treated for 4 hr with up to 2 mg/ml without S9 and with about 0.6 mg/ml (limit of solubility) with S9.	Mutation	With and without S9	? Slight indication of an effect seen in the presence of S9, but there was no dose response. Considered "indeterminate" by Cameron et al. and -ve by CalEPA, 1999.	Cameron et al. 1987
Hamster fibroblast cells	No details available in the expert review.	Cell transformation	With and without S9	-ve	Longstaff et al. 1984
Rat embryo cells	Cells treated for 4 days with up to 10 µg/ml and examined for cell transformation.	Cell transformation	Without	+ve	Price et al. 1978
Rat hepatocytes	Cells treated for 4 hours with up to $1x10^{-3}$M (toxic concentration)	DNA damage	Not applicable	-ve	Kornbrust & Barfknecht, 1985

	and DNA repair assessed.				
Salmonella typhimurium strains TA97, TA98, TA100, TA1535, TA1537	Ames assay. Tested in a preincubation assay at concentrations of up to 10 mg/plate.	Mutation	With and without rat and hamster liver S9	Weakly +ve or +ve in TA98, TA100 and TA1537 in presence of rat liver S9. A good quality study.	NTP, 1983 & 1984
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	Ames assay. Tested at concentrations of up to 10 mg/plate.	Mutation	With and without rat and hamster liver S9	-ve A good quality study.	Cameron et al. 1987
Salmonella typhimurium, including strains TA92, TA94, TA98, TA100, TA1535, TA1537, TA1538	Ames assay. Tested at concentrations of up to 10 mg/plate.	Mutation	Tests included both with and without S9	-ve	Auletta et al. 1977; Bonin & Baker, 1980; Brown et al. 1978; Gubbini et al. 1975; Ishidate et al. 1980, 1984 & 1988; Kawachi et al. 1980 & 1981; Longstaff et al. 1984; Ozaki et al. 1998; Tarján & Kürti, 1982a & b; Yamada et

					al. 1988
Salmonella typhimurium, strains TA97, TA102	Ames test. Tested at concentrations of up to 10 mg/plate. Paper in Japanese with the abstract and data tables in English only.	Mutation	With and without S9	weakly +ve in TA102 with S9, -ve without A limited assay as only tested in 2 strains.	Fujita & Sasaki, 1993
Salmonella typhimurium strains TA98, TA100 and TA1537	Host-mediated assay. Abstract only published and no further details given	Mutation	Intact mouse	-ve	Tarján & Kürti, 1982a & b
Salmonella typhimurium, strains TA1535 and TA1538 and *Escherichia coli*, strain WP2 uvrA	Fluctuation assay for mutation (non-standard method). Tested at a concentration of 1 mg/ml.	Mutation	With and without S9	-ve A limited assay.	Haveland-Smith & Combes, 1980
Escherichia coli, strain not known	Tested at a concentration of 5 mg/ml. No further details available in the citing source.	Mutation	Presumably without	-ve A limited assay.	Lück & Rickerl, 1960
Escherichia coli, strain K-12	No details available from the very brief and unclear	Mutation	Presumably without	-ve (?) The investigators	Karplyuk et al. 1984

	English abstract of this [presumably] Russian publication.			concluded that "the doses of these dyes applied in food industry are fairly safe".	
Streptomyces coelicolor, *Aspergillus nidulans*	No details available from the English translation of a brief Italian abstract.	Mutation	Not known	-ve No independent interpretation of these poorly presented data is possible.	Gubbini et al. 1975
Bacillus subtilis, strains H17A, M45T	DNA repair (rec) assay measuring differential killing. Tested at concentrations of 5 mg/disc, [presumably] 1 mg/ml and, apparently, 2 mg [presumably 2 mg/ml]. In one of the papers, only the data tables are in English, the remainder of the paper being in Japanese.	DNA damage (indicative test)	Possibly with and/or without	No conclusion possible as no inhibition zone seen.	Fujita et al. 1976; Kada et al. 1972; Ozaki et al. 1998
Bacillus subtilis, strain not known	DNA repair (rec) assay. No further details available from the citing source.	DNA damage (indicative test)	With and without	-ve The extent of the zone of inhibition not known.	Kawachi et al. 1980 & 1981; Mizuta & Umisa, 1979; Tonogai et

					al. 1979
Bacillus subtilis, strains H17, M45; H17, M45T	DNA repair (rec) assay, spot test. No further details available from the GENETOX citing source.	DNA damage (indicative test)	Not known	"No conclusion" [Presumably no inhibition zone seen.]	Leifer et al. 1981
Escherichia coli, strains WP2 trp uvrA, WP67 trp uvrA polA, WP100 trp uvrA recA	DNA repair (rec) assay. Tested at a concentration of 1 mg/ml.	DNA damage (indicative assay)	With and without S9	-ve The extent of the zone of inhibition is unknown.	Haveland-Smith & Combes, 1980
Saccharomyces cerevisiae yeast, strain BZ 34	Cells incubated for 4hr with a concentration of 5 mg/ml and examined for gene conversion.	Gene conversion	Without	-ve	Sankaranarayanan & Murthy, 1979
Saccharomyces cerevisiae yeast, strain not known	No details available from the GENETOX citing source.	Mitotic recombination or gene conversion	Not known	-ve	Zimmermann et al. 1984

References

Au W & Hsu T C (1979). Studies on the clastogenic effects of biologic stains and dyes. Environmental Mutagenesis 1, 27-35.

Auletta A E et al. (1977). Lack of mutagenic activity of a series of food dyes for Salmonella typhimurium. Mutation Research 56, 203-206.

BIBRA (1995). Indigo carmine. Toxicity Profile, P226. BIBRA Information Services Ltd, Sutton, Surrey, UK.

Bonin A M & Baker R S U (1980). Mutagenicity testing of some approved food additives with the salmonella/microsome assay. Food Technology in Australia 32, 608-611.

Brown J P et al. (1978). Mutagenicity testing of certified food colors and related azo, xanthene and triphenylmethane dyes with the Salmonella/microsome system. Mutation Research 56, 249-271.

Cal/EPA (1999). Availability of draft data summaries and draft priorities for chemicals with respect to their potential to cause cancer. Available via: http://www.oehha.ca.gov/prop65/docs_state/btch3ntc.html on the internet.

Cameron T P et al. (1987). Mutagenic activity of 27 dyes and related chemicals in the Salmonella/microsome and mouse lymphoma TK+/- assays. Mutation Research 189, 223-261.

Das S K & Giri A K (1988). Chromosomal aberrations induced by secondary and tertiary amine-containing dyes and in combination with nitrite in vivo in mice. Cytobios 54, 25-29.

Durnev A D et al. (1995). Analysis of the cytogenetic activity of food dyes. Voprosy Meditsinskoi Khimii 41, 50-53.

Fujita H & Sasaki M (1993). Mutagenicity test of food additives with Salmonella typhimurium TA97 and TA102. Annual Report of the Tokyo Metropolitan Research Laboratory of Public Health 44, 278-287.

Fujita H et al. (1976). Mutagenicity of dyes in the microbial system. Tokyo Toritsu Eisei Kenkyusho Nempo (Annual Report - Tokyo Metropolitan Research Laboratory of Public Health) 27, 153-158.

GENETOX (1995). Peer-reviewed mutagenicity test data, record on indigo carmine, last revision date 06 April 1995. Record accessed 04 February 2004.

Giri A K & Mukherjee A (1990). Sister chromatid exchange induced by secondary and tertiary amine containing dyes and in combination with nitrite in vivo in mice. Cancer Letters 52, 33-37.

Giri A K et al. (1986). Effects of dyes indigo carmine, metanil yellow, fast green FCF and nitrite in-vivo on bone marrow chromosomes of mice. Cancer Letters 30, 315-320.

Gubbini L et al. (1975). Mutagenicity testing of environmental chemical dyes. Atti Associazione Genetica Italiana 20, 43-44.

Haveland-Smith R B & Combes R D (1980). Screening of food dyes for genotoxic activity. Food and Cosmetics Toxicology 18, 215-221.

Ishidate M Jr (1987). Chromosomal aberration test in vitro. L.I.C., Inc., Tokyo (cited in Ishidate et al. 1988).

Ishidate M et al. (1980). Hen'igen to Dokusei 3 82.

Ishidate M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22, 623-636.

Ishidate M et al. (1988). A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell culture. Mutation Research 195, 151-213.

JECFA (1975). Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain food additives. Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 6.

Kada T et al. (1972). In vitro and host-mediated "rec-assay" procedures for screening chemical mutagens: and phloxine, a mutagenic red dye detected. Mutation Research 16, 165-174.

Karplyuk I A et al. (1984). Study of the mutagenic action of the food dyes, tartrazine and indigo carmine. Voprosy Pitan. 2, 58-61.

Kawachi T et al. (1980). Results of recent studies on the relevance of various short-term screening tests in Japan, in: the predictive value of short-term screening tests in carcinogenicity evaluation. Applied Methods in Oncology 3, 253-267 (cited in BIBRA, 1995).

Kawachi T et al. (1981). No title provided. IARC Scientific Publication No. 27, p. 326 (cited in BIBRA, 1995).

Khera K S & Munro I C (1979). A review of the specifications and toxicity of synthetic food colors permitted in Canada. CRC Critical Reviews in Toxicology 6, 81-133.

Kornbrust D & Barfknecht T (1985). Testing of 24 food, drug, cosmetic and fabric dyes in the in vitro and the in vivo/in vitro rat hepatocyte primary culture DNA repair assays. Environmental Mutagenesis 7, 101-120.

Leifer Z et al. (1981). Evaluation of tests using DNA-repair deficient bacteria for predicting genotoxicity and carcinogenicity: report of the U.S. EPA's GENE-TOX program. Mutation Research 87, 211-297 (cited in GENETOX, 1995).

Longstaff E et al. (1984). No title provided. Dyes Pigments 5, 65-? (cited in BIBRA, 1995).

Lück H & Rickerl E (1960). Lebensmittelzusatzstoffe und mutagene Wirkung. VI. Mitteilung Profung der in Westdeutschland zugelassenen und ursprunglich vorgeschlagenen. Lebensmittelfarbstoffe auf mutagene Wirkung an Escherichia coli. Zeitschrift für Lebensmittel Untersuchung 112, 157 (cited in JECFA, 1975; Khera & Munro, 1979).

Mizuta M & Umisa H (1979). Screening of food dyes by spore rec-assay: effect of the addition of saccharides on mutagenic action. Kenkyo Hokoku-Hiroshima-ken Eisei Kenkyusho 26, 34-38 (cited in BIBRA, 1995).

NTP (1983). NTP studies on CI acid blue 74. Genetic toxicity studies: in vitro study data: Salmonella. Study ID 190683. National Toxicology Program (http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm).

NTP (1984). NTP studies on CI acid blue 74. Genetic toxicity studies: in vitro study data: Salmonella. Study ID 999076. National Toxicology Program (http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm).

Ozaki A et al. (1998). Mutagenicity and DNA-damaging activity of decomposed products of food colours under UV irradiation. Food and Chemical Toxicology 36, 811-817.

Price P J et al. (1978). In vitro and in vivo indications of the carcinogenicity and toxicity of food dyes. International Journal of Cancer 21, 361-367.

Sankaranarayanan N & Murthy M S S (1979). Testing of some permitted food colours for the induction of gene conversion in diploid yeast. Mutation Research 67, 309-314.

Sasaki Y F et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutation Research 519, 103-119.

Tarján V & Kürti M (1982a). Mutagenicity testing of several food colorants certified for use in Hungary. Mutation Research 97, 228.

Tarján V & Kürti M (1982b). Egeszsegtudomany 26, 111.

Tonogai Y et al. (1979). Studies on the toxicity of coal-tar dyes II. Examination of the biological reaction of coal-tar dyes to vital body. Journal of Toxicological Sciences 4, 211-219 (cited in BIBRA, 1995).

Yamada J et al. (1988). Enhancing effect of indigocarmine on the mutagenicity of Trp-P-1 in the Salmonella/microsome. Agriculture and Biological Chemistry 52, 2893-2896.

Zhurkov V S (1975). Investigation of the mutagenic activity of drug preparations and food additives in a culture of human lymphocytes. Soviet Genetics 11, 528-530.

Zimmermann F K et al. (1984). Testing of chemicals for genetic activity with Saccharomyces cerevisiae: a report of the U.S. Environmental Protection Agency GENE-TOX program. Mutation Research 133, 199-244 (cited in GENETOX, 1995).