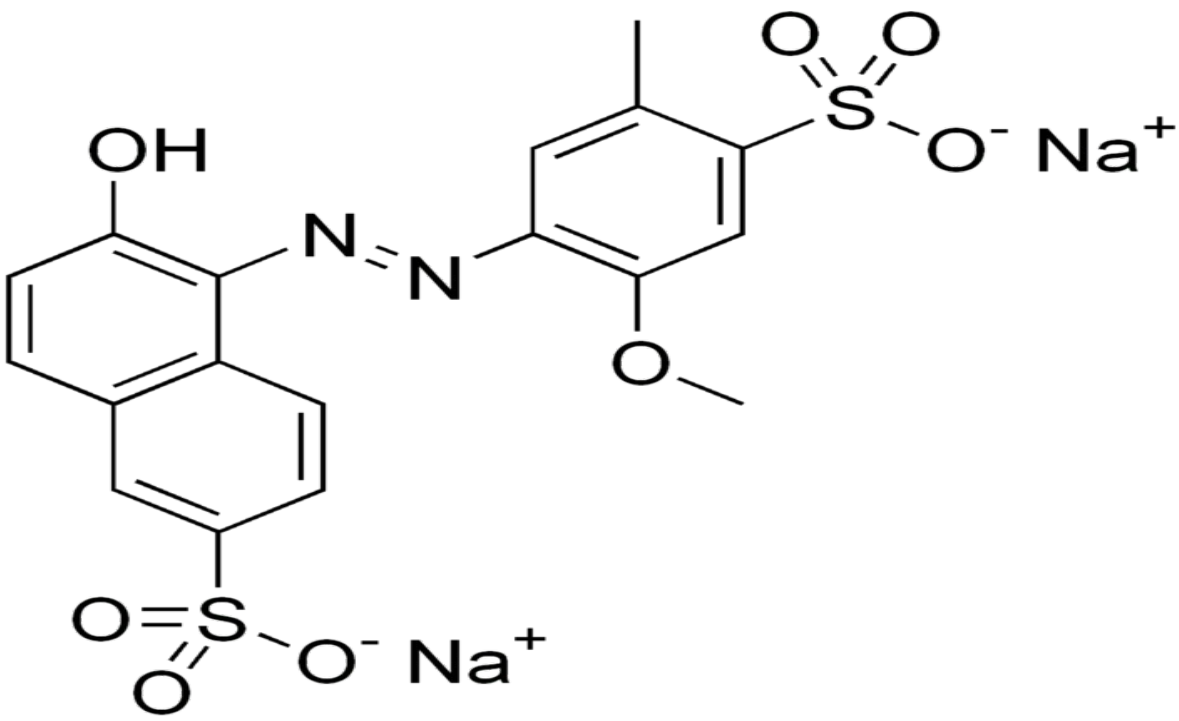
Allura Red E 129



Index de referinţă al culorii: rosu alimentar #17
Statut u.s.a. (f.d.a.): f.d.& c rosu #40
Statut c.e.e.: E 129
C.a.s. no.: 25956-17-6
C.I. - 16035
Conţinutul minim de culoare: 85%.

Properties	
Molecular formula	$C_{18}H_{14}N_2Na_2O_8S_2$
Molar mass	496.42 g mol^{-1}
Appearance	dark red powder
Melting point	>300 °C

Specificatii limite:
- materii volatile & sare: < 15.00 max.
- materii insolubile în apã: < 0.20 max.
- extract de eter: < 0.20 max.
- sulfaţi:
- coloranţi adiţionali:
a)bază leuco:
- materii subsidiare< 3.00 max.
- intermediari sintetici< 0.50 max.

- amine aromatice primare nesulfonate< 0.01 max.
- plumb< 10.00 ppm max.
- arsenic< 3.00 ppm max.
- cadmiu < 1.00 ppm max.
- cupru
- crom
- mercur < 40.00 ppm max.
- fier
- metale grele < 40.00 ppm max.
- diluție max. 5% în apă
- cantitatea maxim admisă conform o.m.s.: 975 / 1998 mg/kg
- grupele 7,8,9,10 max. 70 rahat max. 100
- grupele 11,13,14,16
- grupa 10 max. 70.

Interzisă folosirea în industria cărnii (grupa 3) şi a pastelor făinoase!

Termen de valabilitate: 36 luni de la data producției înscrisă pe etichetă

Condiții de depozitare: în mediu uscat, în ambalajele originale bine închise, ferite de căldură şi lumină, în condiții normale de depozitare (5-25 grd C).

Allura Red AC (E 129) is an azo dye allowed as a food additive in the EU that has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and the EU Scientific Committee for Food (SCF) in 1984 and 1989. Both committees have established an Acceptable Daily Intake (ADI) of 0-7 mg/kg body weight (bw)/day.

Allura Red is a synthetic acid dye containing both NN and CC chromophore groups (pyrazolone dye); dark red powder; soluble in water; melting point 300 C. used in coloring food, cosmetics and meications (FD & C Red No.2). The chemical designation is 2-hydroxy-1-(2-methoxy-5-methyl-4-sulfonato-phenylazo) nephthalene- 6-sulfonate, disodium salt. Acid dyes are water-soluble dyes employed mostly in the form of sodium salts of the sulfonic or carboxylic acids. They are anionic which attach strongly to cationic groups in the fibre directly. They can be applicable to all kind of natural fibres like wool, cotton and silk as well as to synthetics like polyesters, acrylic and rayon. But they are not substantive to cellulosic fibres. They are also used in paints, inks, plastics and leather.

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of Allura Red AC (E 129). Allura Red AC has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and the EU Scientific Committee for Food (SCF) in 1984 and 1989. Both committees established an Acceptable Daily Intake (ADI) of 0-7 mg/kg body weight (bw)/day. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since

then and the data available following a public call for data. New studies included a study by Tsuda et al. from 2001 reporting effects on nuclear DNA migration in the mouse in vivo Comet assay, and a study by McCann et al. from 2007 that concluded that exposure to a mixture including Allura Red AC, resulted in increased hyperactivity in 8- to 9-years old children. The Panel notes that Allura Red AC was negative in in vitro genotoxicity as well as in long-term carcinogenicity studies and that the effects on nuclear DNA migration observed in the mouse in vivo Comet assay are not expected to result in carcinogenicity. The Panel also concurrs with the conclusion from a previous EFSA opinion on the McCann et al. study that the findings of the study cannot be used as a basis for altering the ADI. The Panel concluded that the present database does not give reason to revise the ADI of 7 mg/kg bw/day. The Panel also concludes that at the maximum reported levels of use refined intake estimates are generally below the ADI, although in 1-10 years old children the high percentile of exposure (95th) can be slightly higher than the ADI at the upper end of the range.

ALLURA RED AC

Explanation

This food colorant was first evaluated by JECFA in 1974 (see Annex I, Ref. 34). At that time the Committee decided not to set an ADI for this substance because of a lack of metabolism studies and the unsatisfactory nature of the only long-term study in rats available for evaluation; too few animals remained at the end of the study to allow a satisfactory assessment to be made. Additional studies in these areas were found essential to be carried out before a definite evaluation could be made. A short monograph was published (see Annex I, Ref. 35). The substance was re-evaluated subsequently when some metabolic studies using radio-labelled materials were made available (see Annex I, Ref. 43). These studies demonstrated that the metabolism of this colour was similar to that of other members of the azo dye class. No monograph was prepared and no acceptable daily intake (ADI) was allocated. JECFA in 1979 had again re-evaluated this compound (see Annex I, Ref. 51).

Since the previous evaluations additional data have become available and are summarized and discussed in the following monograph. The previous monograph has been expanded and is reproduced in its entirety below.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

Rats were fed a diet containing 5.19% of Allura Red (White, 1970). It was observed that 0.1% and 29% of the intact dye was excreted in the urine and faeces respectively. In later studies, rats and dogs were pretreated daily with nonradioactive Allura Red. Subsequently, the animals were dosed with the 35S labelled compound and studied for up to 72 hours for excretion and distribution patterns of the colour. Both species showed limited absorption of the compound with the major route of excretion being via the faeces. In the dog 92 to 95% of the recovered radioactivity appeared in the faeces within 72 hours while in the rat 76 to 92% of the recovered radioactivity appeared in the faeces within this time period. Urinary recoveries of the colour in rats and dogs, respectively varied between 5.7 and 19.8% and 2.7 and 3.6%. After sacrifice, significant retention of radioactivity was located in the intestinal contents of both species and in the washed intestines of the rats. This was thought to be due to adhesion of the compound to the intestinal wall, since the total carcass and viscera of these animals contained less than 0.4% of the administered dose (Guyton & Reno, 1975).

Cresidinesulfonic acid was found to be the major metabolite of Allura Red in the urine of these two species, whereas the parent compound was not measurable. In addition, two other unidentifiable

metabolites were found in the urine of the rats. In the rat faecal extracts, cresidinesulfonic acid was a major metabolite along with two unknowns and the parent compound. The dog faecal sample revealed an identical metabolite pattern as seen in the rat, and in addition, a third unknown was discovered. One of the urinary unknowns demonstrated an Rf value which was identical to that of the one of the faecal unknowns suggesting that they were one and the same. The other unknowns exhibited distinctive Rf values which indicated that these metabolites were different (Guyton & Stanovick, 1975).

It has been postulated that azo reduction by gut flora of the dye will yield the two components of the parent compound:

2-methoxy-5-methyl-aniline-4-sulfonic acid (cresidine-4-sulfonic acid)

and 1-amino-2-naphthol-6-sulfonic acid

(White, 1970)

It appears that negligible quantities of intact Red are absorbed and excreted in the urine, and that the major portion of the colour is excreted as metabolites in the faeces.

TOXICOLOGICAL STUDIES

Reproduction

Rat

Groups of 10 male and 20 female rats received 0, 0.37, 1.39 or 5.19% of Allura Red in their diet through two parental (F1A was the P2 generation) and two filial generations. Mating occurred after 27 weeks on either the control or test diets for both the P1 and P2 generation. The fertility indices were low for the controls and test animals in the F1A and F1B generation as well as in the low test level F2A and all test levels of F2B generation. Growth was suppressed slightly for the lowest test level in F1B and high test levels in F1A and F1B as well as for the high test levels in F2A and F2B pups. Other indices, litter size and pup weight at 24 hours, were comparable in each group in each generation. No consistent pathological changes were noted in the P1, F1A, F1B and F2B generations. No evidence was seen of teratogenic or embryotoxic effects regarding implantation sites, resorption sites and live foetuses indices. No difference from controls was noted with regard to appearance, anatomy and structure of test foetuses (Blackmore et al., 1969).

Teratogenicity

Rat

Groups of 24, 19, 20, 21 and 16 pregnant rats received respectively 0, 15, 30, 100 and 200 mg/kg of the dye by gavage daily during pregnancy days 0-19. Gross observations indicate no dye-induced effects in terms of early or late deaths, resorptions per litter, pre-implantation loss, number of foetuses per litter and average foetus weight (Collins, 1974).

Rabbit

In three groups of 14 rabbits, Allura Red was given in doses of 0, 200 and 700 mg/kg bw by gavage from day 6 to 18 of pregnancy. There were no indications of compound-related effects with regard to appearance and behaviour, body weight or in gross necropsy findings for the maternal dose. No adverse effects on implantation and litter

data were noted nor were any foetal abnormalities observed (Reno, 1974).

Mutagenicity studies

Microbial assay systems (plate and suspension tests) with and without the addition of mammalian metabolic activation enzymes were used to determine the mutagenic potential of Allura Red. One strain of yeast, Saccharomyces cerevisiae, and five strains of the bacteria

Salmonella typhimurium were used in the study which employed negative and positive controls. Preliminary toxicity studies were conducted and the compound was found to be nontoxic at the 5% concentration. Allura Red did not exhibit genetic activity in any of the assay systems employed (Brusick, 1976).

Genetic tests were also conducted using three strains of Saccharomyces cerevisiae with and without liver enzyme induction (Anonymous, 1977a). The colour also proved to be negative in these systems.

The Salmonella/microsome system was used to test a number of azo dyes including FD&C Red No. 40. The dye, as well as its chemically reduced component amines, were tested with five tester strains (TA 1535, TA 100, TA 1537, TA 1538 and TA 98). In addition, the effects of the colour were analysed in the same five tester strains with microsomal activation (S-9) and in an aerobic liquid test with microsomal activation. None of these test systems demonstrated mutagenic activity of FD&C Red No. 40 (Brown et al., 1978).

In a second Salmonella/mammalian-microsome study, the colour was tested for mutagenic potential in two frame-shift histidine (TA 1537 and TA 98) and two base-pair substituted histidine mutants (TA 1535 and TA 100). Both the spot test and the plate incorporation assay, with and without the S-9 mix, were employed in these experiments. As before, Allura Red was found to have no mutagenic effects in these assay systems (Muzzall & Cook, 1979).

The same four strains of Salmonella typhimurium (TA 1535, TA 1538, TA 100 and TA 98) with and without microsomal activation were used in the spot and plate tests to study the effects of Allura Red. None of the tests performed demonstrated either a mutagenic or cytotoxic activity of this compound (Viola & Nosotti, 1978).

A rat hepatoma cell culture system was utilized to determine if the colour had enzyme induction capabilities, and it was found that

the colour could not induce aryl hydrocarbon hydroxylase activity (Bradlaw, 1979).

Allura Red was tested in the genetic analysis for recessive lethal effects by the oral feeding to Drosophila melanogaster at the LD50 dose for 24 days. The following genetic tests were conducted: loss of X or Y chromosome, visible mutation at specific loci and sex-linked recessive lethal damage in both mature and immature spermatozoa, chromosomal translocation, sex-linked lethal damage of aged-in-the female spermatozoa and sex-linked mosaic recessive lethal damage in mature spermatozoa. There was no significant increase in the proportion of mutation in any category when comparisons were made with the individual controls. However, when the controls were combined with the controls used in two other studies, a significant increase was seen for the category of sex-linked mosaic recessive lethal damage (Anonymous, 1977b, 1978).

The heritable translocation potential of Allura Red was analysed in eight to 10-week-old male mice who were fed diets containing the colour at dose levels of 4000 and 20 000 ppm for eight weeks. Each male was mated with two females to produce an F1 generation. The males in later generations were mated and their reproductive performance was analysed. The administration of the colour did not affect the fertility of the treated mice. Although several of the animals receiving the colour were potential translocation heterozygotes, cytogenetic analysis showed that these animals were normal. It was concluded that the compound was negative with respect to induction of inheritable translocation in mice (Jorgenson et al., 1978).

Acute toxicity studies

Animal	Route	LD50 (mg/kg bw)	Reference
Rat	Oral (gavage)	10 000	Weir, 1965a
Rabbit	Dermal	10 000	Weir, 1967
Dog	Oral (gavage)	5 000	Weir, 1965b

Rat

The colour was administered to six groups (five animal/sex) at doses varying from 215 to 10 000 mg/kg. The only compound-related effect of note was the red coloration of the urine and faeces (Weir, 1965a).

Skin irritation and sensitization studies

Rabbit

Tests for dermal irritation on intact and abraded skin showed no gross irritation at levels of 0.316, 1.0, 3.16 and 10 g/kg bw of Allura Red; none the less a persistent skin staining did occur (Weir, 1967).

Daily application of the colour at rates of 0.5 g/kg, five days a week for 15 applications on abraded and 65 applications on intack skin of rabbits, of 0.1 and 1% solutions in water or as a hydrophilic ointment revealed no compound-related effect on general appearance,

body weight, clinical laboratory studies and gross and microscopic pathology. No dermal irritation was noted, except that produced by the hydrophilic ointment base (Blackmore, 1968).

Human

Prophetic patch test

Allura Red was applied either as a neat or as a 25% aqueous solution to the skin of 200 human subjects. The initial exposure to the compound was for 72 hours, and this was followed by a 24-hour application 10 to 14 days later. None of the subjects exhibited compound induced irritation or sensitization (Osbourn, 1972).

Draize-Shelanski repeated insult patch test

In this study, the colour and its alumina lake were applied to the subjects volvar forearms (200 subjects) as an aqueous solution for 10 alternate days, for 24-hour periods, followed by a 14-day rest period. Challenge batches were then applied under occlusion to fresh skin sites on the subjects scapular backs for 24 hours. The colour did not produce either irritation or allergic responses during the induction phase nor contact dermatitis in the challenge period (Jolly, 1973).

Photosensitization potential test

As in the previous study, Allura Red and its lake were evaluated on sites under occlusion for five 48-hour, alternate-day periods. These sites had been previously irradiated for five minutes with Xenon light which had been filtered through a window-glass equivalent to limit the exposure to non-erythema-producing, long-wave radiation. A 10-day rest period followed this induction exposure, and then the colour was applied to fresh skin sites, irradiated for five minutes with Xenon and subsequently removed and the sites were evaluated. Allura Red was shown not to produce photosensitization on the 25 subjects studied (Jolly, 1973).

Hypersensitivity reactions

In 52 patients who were suffering from urticaria or angioedema for more than four weeks were placed on an elimination diet for at least three weeks. All non-vital drugs were suspended during the study as were any food ingredients which were known to cause urticaria. In most patients, challenge with Allura Red was performed when the patients were free of symptoms. Reactions were considered positive only when the flare-up of symptoms was reproducible within the same time from intake to skin reaction. Allura Red was administered via the oral route in either a dose of 1 or 10 mg, and induced a positive reaction in 15% of the 52 patients challenged (Mikkelsen et al., 1978).

Short-term studies

Rat

Groups of 10 male and 10 female rats were fed diets containing 0, 0.37, 0.72, 1.39, 2.69 and 5.19% of Allure Red for six weeks. No evidence of compound-related effects were noted as regards body weight, food consumption, survival, organ weights, gross and microscopic pathology. Haematology and urinalysis were normal and no evidence of Heinz body formation was noted (Weir & Crews, 1966a).

Dog

Four groups of one male and one female beagle dogs received Allure Red daily in capsule form at the following doses, 0, 125, 250 and 500 mg/kg bw. No adverse effects were noted on body weight, food consumption, survival, organ weights, gross and histopathology, haematology and clinical chemistry. At the highest level, there were slight ill-defined hepatic parenchymal changes in both sexes (Weir & Crews, 1966b).

Groups of four male and four female beagle dogs received 0, 0.37, 1.39 and 5.19% of Allure Red in their diet for 104 weeks. All animals remained normal regarding appearance, behaviour, haematology and clinical chemistry findings and gross and histopathology. Both faeces and urine were coloured at all test levels. At 52 weeks, an interim sacrifice was performed and revealed that the adrenal cortical cells of the high level groups showed some vacuolation. There was brown pigment deposition in the Kupffer cells of females at the two lower test levels. These changes had disappeared by 104 weeks and special histological examination of the eyes revealed no adverse changes (Olson et al., 1970).

Pig

SPF pigs of Danish Landrace were equilibrated for a period of 21 days, whereupon they were distributed according to body weight into nine groups which consisted of two males and two females. The compound was administered by gavage three hours after feeding in the morning. The dye was administered at a dose of 1000 mg/kg/day for the first 21 days, thereafter the dose was increased to 1500 mg/kg/day and this dosage was given for an additional 54 days. Body weight was recorded weekly and food intake daily. Blood samples were collected two days prior to and five, 19 and 68 days following the initiation of colorant administration. The haematological and clinical parameters measured were haemoglobin, packed cell volume, total erythrocyte count, counts of Heinz bodies and reticulocytes and serum activities of LDH and isoenzymes. Autopsy and histological examination of kidneys, spleen, liver, hepatic and renal lymph nodes and bone marrow were performed. The colour produced no significant effects on clinical and haematological parameters nor did it elicit any observable pathological changes (Sondergaard et al., 1977).

Long-term studies

Mouse

A control group of 50 male and 50 female mice, a positive control group of 25 male and 25 female and a test group of 50 male and 50 female mice were treated dermally with 0.1 ml of either distilled water, 10 µg 3,4-benzypyrene in acetone or a 5% Allura Red test solution twice weekly for 20 months. The results in the positive control group showed the mouse strain used to be sensitive to benzypyrene carcinogenesis. Survival, gross and histopathology of major organs were comparable in the negative controls and animals treated with Allura Red. Histology of the skin revealed comparable

incidence and degree of severity of acanthosis, hyperkeratosis and dermatitis for the negative control and the Allura Red groups (Voelker, 1970).

Groups of 50 CD(R)-1 albino mice/sex received Allura Red in their diets at levels of 0.37, 1.39 and 5.19%. These animals were the offspring of parental mice treated at identical levels for one week prior to breeding and through gestation and lactation. An additional group of 50 animals/sex served as a negative control group. An interim sacrifice was performed at 42 weeks which resulted in reducing the number of animals in each group and sex to 30. This was done to determine compound effects upon the early onset of tumours of the lymphatic system. Prior to this, neoplasms which were histologically diagnosed as lymphomas were found in several animals which received the colour. Statistical analysis of the incidence of tumours occurring throughout the study did not reveal any increase in tumour incidence due to compound administration. No treatment-related effects were noted in the survival, clinical signs and clinical laboratory data. The animals on the highest dosage level demonstrated lower body weights and effects on organ weight data and organ/body weight ratios, however, the meaning of these observations ware considered to be inconsequential. Gross and microscopic examination of all the animals revealed an absence of treatment-related effects and spontaneous disease-related lesions were considered to be within acceptable limits (Serota et al., 1977a).

A second lifetime dietary mouse study was performed using a protocol identical to the one described above, with the exception that 100 animals/sex/group and two negative control groups were used. As before, statistical evaluation of neoplasms of the reticuloendothelial system failed to show a significant correlation between tumour response and increasing dosage. Sporadic changes were noted in the clinical signs, body weight, food consumption and survival rates; none the less, these could not be related to the administration of the colour. Alterations of the absolute and relative weights of the adrenals and thyroids were noted in several of the treatment groups. Histological examination of these tissues in the animals in the highest dose group at the completion of the study demonstrated no abnormalities. Therefore, the significance of weight gains in these

two organs could not be established. Generally, gross pathological findings were similar in the control and treated animals. There was, however, a higher incidence of dark areas in the lungs of those animals in the high dose group which died or were sacrificed in extremis and of cystic foci areas in the kidneys of the high dose males. No distinct treatment-related effects could be ascribed to

these findings. Histopathological evaluation did not show effects which could be attributed to compound administration, nor was there evidence for a dose-related increase in the incidence of spontaneously occurring tumours (Reno et al., 1978).

Rat

Groups of 30 male and 30 female rats received in their diet 0, 0.37, 1.39 and 5.19% of Allura Red for 92 weeks. Moderate growth depression occurred at the 5.19% level in both sexes. No other compound-related effects were noted as regards appearance, behaviour, survival, organ weights, clinical laboratory studies or gross and histopathology. No evidence of Heinz body formation was noted apart from a slight tendency to anaemia (Olson & Voelker, 1970).

50 CD(R)-1 albino rats/sex were placed in a negative control group and three test groups which contained varying percentages (0.37, 1.39 and 5.19) of Allura Red. The males were administered the diets for 118 weeks, and the females were fed for 121 weeks. All of these animals were the F1a offspring of parental rats which were treated at identical levels for one week prior to breeding, through a three-week breeding period and during the gestation and lactation periods. No treatment-related effects were noted in comparisons of survival, clinical signs, clinical laboratory data and organ weight data. The mean body weights and growth rates of the animals in the high-dose group were lower than those of control animals. However, the low dose group exhibited greater mean food consumption values than the control groups. Gross pathological and histopathological findings were essentially comparable between the control and treated groups. However, increased incidences of kidney discoloration and firm granular material in the pelvis of the kidney were noted in the males of the colour treated groups. A slightly higher incidence of granules in the pelvis was also noted in the high-dose males sacrificed at termination. However, no significant dose-related trend was noted in the statistical analysis of these data. An increased incidence of renal calculi and focal epithelial proliferation was noted in the high-dose rats, but additional examination of the other groups indicated that this toxic effect could not be attributed to the administration of the compound (Serota et al., 1977b).

Comments

Allura Red when administered orally undergoes partial azo reduction prior to absorption. Metabolic studies indicate that the colour is poorly absorbed in the body, and the major route of excretion is via the faeces. In a multigeneration reproductive study

on rats it was shown that the progeny of the parents who were fed

51.9 g of the colour per kg of food demonstrated a slight growth depression. The "no effect" level on reproductive physiology of this colour in the rat is 13.9 g/kg of food. Teratogenicity studies in rats and rabbits failed to show any compound-related embryotoxic effects. A variety of mutagenicity studies carried out with Allura Red indicated that there were no mutagenic effects. Another study on acute and short-term oral toxicity of Allura Red in several species revealed that apart from the coloration of the urine and faeces, there were no other compound-related responses. Dermal studies (both short- and long-term) also indicated an absence of colour-induced toxic responses. In long-term feeding studies on mice and rats, the most consistent observation was that the animals that received the greatest amount of colour (51.9 g/kg of food) exhibited lower body weights compared to control animals. One study suggested that mice that were fed on this colour demonstrated an earlier onset of tumours of the lymphatic system compared to control mice.1 However, a second more extensive mouse study has not borne this out. The long-term study and the mutagenicity studies suggest that Allura Red does not possess carcinogenic potential.

The data were sufficient to establish a temporary acceptable daily intake for man pending the availability of the statistical analysis of the long-term mouse study.

EVALUATION

Level causing no toxicological effect

Rat: 1.39% in the diet corresponding to 695 mg/kg bw.

Estimate of acceptable daily intake for man

0-7 mg/kg bw.*

FURTHER WORK OR INFORMATION

Required by 1981

(1) Adequate information of statistical analysis of the long-term mouse studies.

* Temporary.