Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to

2-Isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) in food contact materials

(Question numbers EFSA-Q-2005-240 & EFSA-Q-2005-241)

Adopted on 7 December 2005

SUMMARY

The European Food Safety Authority is asked to carry out risk assessment for substances intended for use in materials in contact with food, according to Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food. In particular, on the basis of Art.29 of Regulation (EC) No 178/2002, EFSA is asked to advise the Commission on the risk for human health of the use of the substances 2-isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) as photoinitiators in inks applied to food packaging materials.

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food noted that inks applied to food packaging materials are not covered by specific European legislation. However, materials and articles intended to come in contact with foods should comply with the general criteria laid down in Art.3 of Regulation (EC) No 1935/2004, i.e. should not transfer their constituents in food in quantities which could endanger human health or bring about unacceptable changes in composition or characteristics of foodstuffs. These criteria are also reiterated in the Council of Europe Resolution AP (2005)2 adopted on 14 September 2005 on printed materials and articles intended to come in contact with food.

Industry reported the results of analytical tests on the occurring levels of ITX and EHDAB in a number of food products packaged in cartons printed with UV-cured inks containing ITX and EHDAB as photoinitiators.
In the milk products intended to be consumed in the first year of life, the level of ITX ranged from 120 to 305 µg/l. The available data (two samples only) on ITX in growing up milk (for aged 12 months on) were 74 and 445 µg/l. ITX was found at 600 µg/l in a single sample of flavoured milk tested. No data on EHDAB levels were reported for these products.
In the milk and soy based products tested, not specifically intended for babies, the level of ITX ranged from 54 to 219 µg/l and the level of EHDAB ranged from 27 to 134 µg/l, for pack sizes of 1000 ml. In a chocolate milk sample (200 ml pack size) ITX was 295 µg/l and EHDAB was 148 µg/l.

In fruit juices, fruit nectars and drinks indicated as “cloudy” due to the presence of fruit pulp and fibres, the levels of ITX ranged from <5 µg/l to 249 µg/l and the levels of EHDAB ranged from <5 µg/l to 125 µg/l. The highest values were reported for smaller pack sizes.
In the fruit juices, fruit nectars, water, and the drinks indicated as “clear”, neither ITX nor EHDAB were detected (detection limit: 5 µg/l).

Exposure assessment was performed by the Panel based on different concentration values. In all exposure scenarios, potential exposure to EHDAB was calculated based on concentration values set at half of the concentration of ITX.
Based on a potential concentration of 250 µg/l of ITX and 125 µg/l of EHDAB in affected milk, potential dietary exposure in infants at the 95th percentile of consumption is respectively 43 µg/kg bw/day and 22 µg/kg bw/day. This level of exposure refers only to infants fed exclusively with liquid infant formulae packaged in cartons printed with UV-cured inks.
For young children, the exposure scenario was based on the hypothesis that half of their food and beverages consumed would be packaged in cartons printed with UV-cured inks. Considering a mixed diet with both milk and fruit juices contributing to packaged foods and beverages, an exposure scenario based on a potential concentration of 125 µg/l of ITX and 63 µg/l of EHDAB would lead to a potential dietary exposure of respectively 12 µg/kg bw/day and 6 µg/kg bw/day in young children at the 95th percentile of consumption. Based on a potential concentration of 250 µg/l of ITX and 125 µg/l of EHDAB assuming that all affected food is milk products only, potential dietary exposure in young children at the 95th percentile of consumption is respectively 23 µg/kg bw/day and 11 µg/kg bw/day.
In the adult, a conservative assumption could be that of the consumption of 3 kg packaged food and beverages each day, half of which being packaged in UV-printed cartons. It leads to an overall potential consumption of 1.5 kg/day of affected products Based on a potential concentration of 250 µg/l of ITX and 125 µg/l of EHDAB in affected products, potential dietary exposure in adults is respectively 6 µg/kg bw/day and 3 µg /kg bw/day. Based on a potential concentration of 125 µg/l of ITX and 62 µg/l of EHDAB in affected products, potential dietary exposure in adults is respectively 3 µg/kg bw/day and 1.5 µg/kg bw/day.
The Panel noted that due to their high consumption of food per kg body weight, infants exclusively fed with infant formulae packed in cartons printed with UV cured inks are potentially more exposed to ITX and EHDAB than other population groups.

ITX was tested with contradictory results in limited genotoxicity studies *in vitro*; however clearly negative results were obtained in two adequate *in vivo* studies. In conclusion, the existing *in vivo* genotoxicity studies do not indicate a genotoxic potential for ITX. No other toxicity data on ITX are available.

In view of the lack of other toxicity data no further comment on the safety of ITX can be made.

EHDAB is not genotoxic and not teratogenic. The NOAEL for general toxicity in 4-week oral studies was 100 mg/kg bw. A large (2500 or greater) margin of safety can be calculated for all exposure scenarios. It is concluded that the occurrence of EHDAB in food from its use in inks applied to food packaging materials is of no safety concern.

KEY WORDS

2-Isopropyl thioxanthone (ITX), CAS 5495-84-1; 2-ethylhexyl-4-dimethylaminobenzoate (EHA, EHDAB), CAS 21245-02-3; 4-Isopropyl thioxanthone; CAS No 83846-86-0; photoinitiators; inks; food contact materials, foods, genotoxicity; toxicology.

BACKGROUND

Inks applied to food packaging materials are not covered by specific European legislation. However, materials and articles intended to come in contact with foods should comply with the general criteria laid down in Art.3 of Regulation (EC) No 1935/2004, i.e. should not transfer their constituents in food in quantities which could endanger human health or bring about unacceptable changes in composition or characteristics of foodstuffs. These criteria are also reiterated in the Council of Europe Resolution AP (2005)2 adopted on 14 September 2005 on printed materials and articles intended to come in contact with food.
A recent notification from the Italian authorities under Art.50 of the Regulation (EC) No 178/2002 on the Rapid Alert System for Food and Feed (RASFF) has shown the occurrence of the ink photoinitiator 2-isopropyl thioxanthone (ITX) in liquid milk for babies packaged in printed carton at a level of 250 µg/l. Later Industry has reported the migration of another ink photoinitiator, 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB), at lower levels than ITX and that also other milk products and cloudy fruit juices packaged in cartons are affected.

ITX is used as a photoinitiator in UV-cured inks, triggering the radical polymerization of the acrylic component of such inks and thus causing the liquid ink film to dry. EHDAB is used as a synergistic agent along with ITX for UV-curable inks. It is also used as a UV filter at up to 8% in cosmetic products.

Following a request from the Commission, Industry has provided information on levels in foods and mutagenicity of ITX and EHDAB, which has been forwarded to the EFSA for evaluation.

TERMS OF REFERENCE

In accordance with Art.29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority (EFSA) is asked to advise the Commission on the risk for human health from the use of the substances 2-isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) as photoinitiators in inks applied to food packaging materials.

ASSESSMENT

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has given consideration to unpublished data provided by Industry on concentration levels and mutagenicity of ITX and EHDAB, as well as to previous opinions on EHDAB expressed by the Scientific Committee on Cosmetic Products and Non-food Products Intended for Consumers (SCCNFP).

Chemistry

2-Isopropyl thioxanthone (ITX), $C_{16}H_{14}OS$, molecular weight 254 Daltons, CAS No. 5495-84-1. ITX is a yellow solid, with melting point 62-77 °C. It is virtually insoluble in water but is soluble in organic solvents, especially non-polar solvents.

2-Ethylhexyl-4-dimethylaminobenzoate (EHDAB), $C_{17}H_{27}O_2N$, molecular weight 277 Daltons, CAS No. 21245-02-3. EHDAB is a yellow liquid that is virtually, insoluble in water but is freely soluble in many organic solvents.

Levels of ITX and EHDAB in foods packaged in cartons

Two unpublished reports on the levels of ITX and EHDAB in foodstuffs packaged in cartons printed with UV-cured inks were provided by Industry: (report A, industry unpublished document, 2005; report B, industry unpublished document, 2005)

Report A

Four types of milk-based food products, packaged in cartons printed with UV-cured inks, were analysed for the levels of ITX. The brands of the food products were not reported. ITX was extracted from the foods with cyclohexane and analysed by gas chromatography coupled with mass spectrometry (GC/MS) with a detection limit of 2 µg/l. Spiking the foods before extraction indicated recovery rates ranging from 85% to 95%. The food samples and the results are reported in Table 1.

Table 1- ITX levels in milk based products

Product	Number of samples	Number of independent lots	ITX (µg/l)
Starter formula*	4	4	120-262
Growing up milk*	9	7	143-305
Growing up milk* (for 12 months on)	2	2	74 and 445
Flavoured milk (200ml volume)	1	1	600

*volumes not reported

Report B

A number of liquid food products packaged in cartons printed with UV-cured inks were analysed for the levels of ITX and EHDAB. The brands of food products were not reported. In the case of milk and soy beverages the extraction of ITX and EHDAB was performed with cyclohexane, after pretreatment with aqueous ammonia and ethanol. In the case of juice and juice beverages, the extraction was performed with diethyl ether. High performance liquid chromatography (HPLC) was used for the analysis, with fluorescence detection for ITX and photo diode array detection for EHDAB. The detection limit was 5 µg/l for both substances. Recovery rates of ITX and EHDAB from spiking milk or orange juice were in the range of 85- 105%.

The results have been reported in three separate groups: milk and soy beverages (Table 2), fruit juices, fruit nectars and "cloudy" drinks (Table 3), fruit juices, fruit nectars and "clear" drinks (Table 4). The fat content of the samples was reported only for milk and soy beverages. According to the authors, the migration of both ITX and EHDAB is higher into fat-containing food products. The high levels measured in some fruit juices, nectars and drinks defined as "cloudy" were rationalised by the authors as due to the presence of citrus oils, fruit pulp and fibres etc. No ITX and EHDAB were detected (detection limit of 5 µg/l) in fruit juices, nectars and drinks defined as "clear"

Table 2- ITX and EHDAB levels in milk and soy beverages, (Report B, industry unpublished document)

Product	**Fat content (%)**	**Pack size (ml)**	**ITX (µg/l)**	**EHDAB (µg/l)**
UHT milk	3.8	1000	142	71
UHT milk	1.5	1000	177	92
UHT milk	0.1	1000	54	27
Soy milk	1.5	1000	219	134
Soy milk vanilla	1.5	1000	170	90
Soy milk & juice	0.6	1000	137	71
Chocolate milk	2.9	200	295	148

Table 3- ITX and EHDAB levels in fruit juices, fruit nectars and "cloudy", drinks (Report B, industry unpublished document)

Product	**Pack size(ml)**	**ITX (µg/l)**	**EHDAB (µg/l)**
Apple/acerola juice	200	45	16
Apricot nectar	200	63	33
Orange juice	200	136	46
Orange juice	200	201	88
Multivitamin drink	200	30	17
Multivitamin juice	200	249	125
Peach nectar	200	95	35
Pear juice	200	70	24
Pineapple juice	200	32	10
Multivitamin drink	330	69	23
Orange & mango nectar	330	117	66
Pineapple & kiwi nectar	330	160	115
Pineapple nectar	330	17	8
Tropical juice blend	330	213	124
Tropical juice drink	330	67	25
Beet root juice	500	32	10
Carrot juice	500	34	12
Mixed vegetable juice	500	57	27

Product	Pack size(ml)	ITX (µg/l)	EHDAB (µg/l)
Tomato juice	500	32	12
Tomato juice	500	38	14
Tomato juice	750	122	68
ACE multivitamin nectar	1000	41	22
Apple juice with fruit	1000	36	26
Banana drink	1000	6	<5
Cranberry, basilicum	1000	27	7
Fruit juice (mix)	1000	47	32
Fruit juice soy drink	1000	25	12
Grape & mango	1000	129	98
Grapefruit juice	1000	33	22
Grapefruit juice	1000	73	36
Mango, red pepper	1000	150	75
Mixed fruit drink	1000	40	13
Orange juice	1000	31	23
Orange & peach nectar	1000	43	27
Orange drink, red	1000	155	73
Orange juice	1000	29	13
Orange juice	1000	45	23
Orange nectar	1000	49	20
Orange, peach drink	1000	< 5	< 5
Orange, pomegranate vitamin drink	1000	187	100
Peach, mango	1000	106	35
Peach nectar	1000	87	34
Pineapple, orange drink	1000	43	17
Soy & fruit (raspberry, rosehip)	1000	75	36
Tomato juice	1000	41	34

Table 4- ITX and EHDAB levels in fruit juices, fruit nectars and "clear" drinks, (Report B, industry unpublished document)

Product	Pack size (ml)	Juice content (%)	ITX (µg/l)	EHDAB (µg/l)
Apple juice	200	100	< 5	< 5
Juice drink	200	15	< 5	< 5
Juice drink	330	30	< 5	< 5
Tea & Juice	330	75% tea/25%juice	< 5	< 5
Water	500	100	< 5	< 5

Product	Pack size (ml)	Juice content (%)	ITX (µg/l)	EHDAB (µg/l)
Apple juice	1000	100	< 5	< 5
Apple juice	1000	100	< 5	< 5
Apple pear drink	1000	6	< 5	< 5
Cherry drink	1000	35	< 5	< 5
Cranberry & Raspberry	1000	21	< 5	< 5
Cranberry drink	1000	25	< 5	< 5
Elder flower drink	1000	22	< 5	< 5
Fruit drink	1000	20	< 5	< 5
Juice/alcohol drink	1000	15% alcohol	< 5	< 5
Red grape juice	1000	100	< 5	< 5
Red grape juice	1000	100	< 5	< 5
White grape & peach	1000	30	< 5	< 5

Evaluation of levels occurring in food

In the milk products intended to be consumed in the first year of life (Table 1), the level of ITX ranged from 120 to 305 µg/l. The available data (two samples only) on ITX in growing up milk (for aged 12 months on) were 74 and 445 µg/l. ITX was found at 600 µg/l in a single sample of flavoured milk tested. No data on EHDAB levels were reported for these products.

In the milk and soy based products tested, not specifically intended for babies (Table 2) , the level of ITX ranged from 54 to 219 µg/l and the level of EHDAB ranged from 27 to 134 µg/l, for pack sizes of 1000 ml. In the chocolate milk sample (200 ml pack size) ITX was 295 µg/l and EHDAB was 148 µg/l.

In fruit juices, fruit nectars and drinks indicated as “cloudy” due to the presence of fruit pulp and fibres (Table 3), the levels of ITX ranged from <5 µg/l to 249 µg/l and the levels of EHDAB ranged from <5 µg/l to 125 µg/l. The highest values were reported for smaller pack sizes.

In the fruit juices, fruit nectars, water, and the drinks indicated as “clear” (Table 4), neither ITX nor EHDAB were detected (detection limit: 5 µg/l).

From the comparison of the results for the different food type groups it appears that food composition is one of the aspects to consider. As expected on the basis of the solubility characteristics of ITX and EHDAB, liquid food products containing fat were more prone to migration of ITX and EHDAB. In the case of non-fatty liquid foods such as fruit juices, fruit nectars and drinks, other factors such as the presence of citrus oils, fruit fibres and pulp, could facilitate the migration of ITX and EHDAB by acting as carriers or co-solvents. In the absence of these two factors, ITX and EHDAB did not migrate to detectable levels.

The information available does not allow identification of other parameters that may influence the level of migration of ITX and EHDAB. It seems likely that the pack size could be a factor since it influences the ratio of package area to food mass.

In Report B, no information was provided on the number of samples for each food product. For both reports, no information was available on the characteristics of the carton package e.g. initial content of photoinitiators, pack shape, age of the packed foods, number of brands and lots considered, conditions of storage, etc. These uncertainties affect the interpretation of the migrational behaviour of these photoinitiators. However, the analytical data provided can be used for a preliminary exposure assessment.

Exposure

Potential dietary exposure to ITX and EHDAB from foods and beverages packed in cartons can arise under conditions where UV offset inks have been used to print the package. Based on lists provided by industry this technology may be used for the following products: products intended for infants including ready-to-drink infant formulae, various milk based and soy based products, fruit juices, liquid creams, desserts, sauces, oils, meal replacements, tomato, rice and yoghurt (industry unpublished document, 2005a).

Potential contamination of the food products increases with fat content and decreases with increasing pack size. Highest concentrations were therefore found in foods packaged in cartons of 200 ml. ITX mainly migrates in fat-containing foods (such as milk and soy based products) but comparable concentration levels have been observed into juices containing citrus and/or with fibre and pulp content (industry unpublished document, 2005b).

According to industry the above mentioned offset printing technology is expensive and used mainly in speciality foods. However, a number of these foods and in particular beverages are typically consumed on a daily basis with many consumers being loyal to a certain brand of milk or of fruit juice. In order to assess exposure in the most highly exposed section of the population, consumers loyal to such products have been considered by the Panel. In particular, brand loyalty may be very high in the case of products intended for infants.

Infants that are not breast fed will be fed infant formula, which is available either in powder form, for home preparation, or as a ready-to-drink formulation packaged in cartons. Exposure to ITX via infant milk may only arise in the case of the latter formulation; no data were available to the Panel regarding the size of the market for this formulation in various countries and hence the potential exposure to ITX. The exposure estimates that follow are based on the worst-case scenario of an infant fed exclusively with a ready-to-drink formula packaged in cartons printed in UV-cured inks.

When detected, concentration values of ITX ranged from 6 to 305 µg/l with two samples above such value (445 µg/l in a growing up milk and 600 µg/l in a flavoured milk). Exposure assessment was performed by the Panel based on different concentration values. The concentration value of 250 µg/l was taken by the Panel as a typical value in affected milk and milk products. The concentration value of 125 µg/l was taken as a conservative estimate of average concentration in the large variety of affected foods and beverages in which ITX was detected, to be used in the exposure scenarios for population groups consuming a varied diet. In infants, an additional exposure scenario was performed by the Panel based on the highest observed value in foodstuffs intended for infants (445 µg/l), due to the very high brand loyalty in this population group. In children and adults such a scenario was not deemed necessary since exposure to ITX may be derived from a variety of products that are not likely to contain always the highest observed levels.

EHDAB concentration data were not available for foodstuffs intended for infants. In other products concentration of EHDAB was typically half of ITX concentration. In all exposure scenarios, potential exposure to EHDAB was calculated based on concentration values set at half of the concentration of ITX.

Potential exposure in infants

The following exposure assessment to ITX and EHDAB refers only to infants fed with liquid infant formulae packaged in cartons printed with UV-cured inks.
A consumption scenario used by Scientific Committeee on Food (European Commission, 2002) to assess infant exposure to substances potentially present in infant formula is that of a liquid consumption of 150 ml /kg bw/day (0.7 l/day in infants aged 0-4 months of estimated average body weight 4.5 kg). The consumption scenario used by industry in the case of ITX is based on the German DONALD study (Kersting, 1998): in a 3 month infant weighing on average 6.1 kg, average and 95th percentile consumption of infant formula (based on a reconstitution ratio of 135 g/l of liquid formula) are respectively 780 and 1060 ml/day, i.e. 128 and 174 ml/kg bw/day.
This last consumption scenario is more conservative and was applied by the Panel in the present opinion. Based on a potential concentration of 250 µg/l of ITX and 125 µg/l of EHDAB in affected milk, potential dietary exposure in infants at the 95th percentile of consumption is respectively 43 µg/kg bw/day and 22 µg/kg bw/day. Based on a potential concentration of 445 µg/l of ITX and 220 µg/l of EHDAB in contaminated milk, potential dietary exposure is respectively 77 µg/kg bw/day and 38 µg/kg bw/day.
The Panel noted that potential exposure could be higher in specific cases such as infants of lower body weight who may have a higher consumption per kg body weight (infants in the first weeks of life and premature infants). On the other hand, potential dietary exposure in older infants decreases with age due to decreasing food consumption per kg body weight and to increasing variety of the diet.

Potential exposure in young children

A large number of beverages consumed by young children are likely to be packed in cartons. In particular, milk based products and fruit juices are likely to be consumed in large quantities by children and are often packed in small volume packages for convenience. Some other foods such as soups, creams and desserts may be packed in the same way. A conservative assumption could therefore be that of a young child having half of his food and beverages packed in UV-printed packages. The 95th percentile consumption of solid and liquid food in young children (1.5-4.5 years) in the UK (2 kg) was used (HMSO, 1995). Based on an average body weigh of 11 kg in a child aged 1.5 years (CEC, 1993), consumption of affected products under this hypothesis would be 91 g /kg bw/day. In order to consider the high contribution of milk to packaged foods and beverages in some young children, an exposure scenario was based on the hypothesis that the 1 kg/day is milk products only. Concentrations of 250 µg/l of ITX and 125 µg/l of EHDAB in affected products would then lead to a potential exposure of 23 µg/kg bw/day and 11 µg/kg bw/day, respectively. Considering a mixed diet with both milk and fruit juices contributing to packaged foods and beverages, another exposure scenario based on concentrations of 125 µg/l of ITX and 63 µg/l of EHDAB in affected foods and beverages, would lead to a potential dietary exposure of 12 µg/kg bw/day and 6 µg /kg bw/day, respectively. The hypothesis that all packaged products would be packed in cartons printed with UV off-set inks should lead to a conservative exposure assessment. Potential dietary exposure in older children should decrease with age due to decreasing food consumption per kg body weight and to increasing variety of foods and beverages consumed. Their exposure is expected to be intermediate between that of young children and adults.

Potential exposure in adults

As noted above for young children, a large number of beverages consumed by adults are likely to be packaged in cartons and some other foods may be packed in this way too. In the same way therefore, a conservative assumption could be that the consumption of packaged food and beverages by adults is 3 kg each day and half is packaged in UV-printed cartons. It leads to an overall potential consumption of 1.5 kg/day of affected products i.e. 25 g /kg body weight considering a 60 kg body weight adult. Based on a potential concentration of 250 µg/l of ITX and 125 µg/l of EHDAB in affected products, potential dietary exposure in adults is 6 µg/kg bw/day and 3 µg/kg bw/day, respectively. Based on a potential concentration of 125 µg/l of ITX and 63 µg/l of EHDAB in affected products, potential dietary exposure in adults is 3 µg/kg bw/day and 1.5 µg /kg bw/day, respectively.

The Panel noted that other food products may also be packaged in materials printed with UV-cured inks.

The above reported preliminary exposure assessments are affected by the uncertainty in the data on the concentration levels in foods. In fact it is not clear whether the data reported by industry may be considered representative of the whole market.
The Panel noted that due to their high consumption of food per kg body weight, infants exclusively fed with infant formulae packed in cartons printed with UV-cured inks are potentially more exposed to ITX and EHDAB than other population groups.

Toxicity

ITX
Five unpublished reports on genotoxicity studies of ITX were provided by Industry:

ITX was tested in a bacterial reverse mutation test with *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2uvrA (iGm resins b.v., 2003a). A sample of the substance (purity not specified, solvent DMSO) was evaluated at 61.7, 185.2, 555.6, 1666.7 and 5000 µg/plate, with and without metabolic activation, using triplicate plates. The study was not conducted according to international guidelines nor was it in compliance with Good Laboratory Practice (GLP). A repeat experiment was not performed.
The results obtained show a dose-related increase in revertant colonies slightly exceeding twice the negative control was observed in strain TA98 at the two highest doses applied, only without metabolic activation. The results with other strains were negative.

ITX was tested in an *in vitro* chromosomal aberration assay in the Chinese hamster cell line CHL/IU (iGm resins b.v., 2003b). The substance (purity not specified, solvent DMSO) was applied for 6 hours with metabolic activation (at 37.5, 70 and 150 µg/ml) and harvested at 24 hours; without metabolic activation, ITX was applied for 6 hours (at 15, 30 and 60 µg/ml) and harvested at 24 hours; in addition, a continuous treatment of 24 hours (with 10, 20 and 40 µg/ml) and 48 hours (with 2.5, 5 and 10 µg/ml) was applied. Structural chromosomal aberrations and polyploidy were scored in two hundred metaphases per experimental point. The study was not conducted in compliance with GLP.
The results obtained show a four-fold increase in structural chromosomal aberrations (2/200 vs 8/200 metaphases) at the highest dose, both after treatment for 6 hours in the presence of metabolic activation, and after treatment for 48 hours without metabolic activation. Due to the lack of information on incidence and type of aberrations in individual cultures, and the lack of

historical control data, these results cannot be properly evaluated. After 48 hour of exposure to ITX, gaps showed a clear increase at all doses (0/200 vs 14/200, 10/200 and 18/200 at 0, 2.5, 5 and 10 µg/ml, respectively): however, the biological significance of gaps is not known.
A sample of ITX (purity not specified, solvent DMSO) was tested in the forward mutation assay at the *tk* locus in mouse lymphoma cells L5178Y (iGm resins b.v., 2003c). Cells were treated for 3 hours with and without metabolic activation (± S9) at the following dose levels: 0, 20, 30, 45, 67.5 µg/ml and seeded for mutant selection after an expression period of 2 days. The study was not in compliance with GLP. A limited protocol, with fewer doses and independent cultures than recommended by the relevant OECD Guideline was applied.
The results obtained show a significant increase in mutant frequency in treated cultures, both without and with metabolic activation. Without S9, ITX induced a dose-related increase in mutants (maximum 3.5-fold) in the dose range 20-45 µg/ml, with relative survival of 47.4 – 22.8 %; the top dose (67.5 µg/ml) was completely cytotoxic. With S9, a 5-fold increase in mutants was observed in the dose range 20 – 67.5 µg/m, with relative survival of 83.5 – 14.2 %. Increased mutant frequencies were associated with an increased incidence of small colonies.

ITX was tested *in vivo* in the mouse bone marrow micronucleus test (Notox, 2004a). The substance (batch 2K40401, purity >98%) was suspended in propylene glycol and administered by single oral intubation to groups of five male NMRI mice at the following dose levels: 0, 500, 1000 and 2000 mg/kg bw (maximum recommended dose). Animals were sacrificed 24 hours after treatment (all dose groups) and after 48 hours (top dose only); micronuclei were scored in 2000 polychromatic erythrocytes (PCEs) per animal. The study was performed according to the relevant OECD guideline and in compliance with GLP.
Treatments were well tolerated, with no signs of systemic toxicity. No increase in the incidence of micronucleated PCEs, and no decrease in the ratio of polychromatic to normochromatic erythrocytes in treated animals compared to vehicle controls were observed. The positive control substance (cyclophosphamide) produced the expected, statistically significant, positive result.

ITX (batch 2K40401, purity >98%) was tested for its ability to induce unscheduled DNA synthesis in rat liver using an *in vivo/in vitro* procedure (Notox, 2004b). Male Wistar rats were dosed once by oral intubation with ITX suspended in propylene glycol at 2000 mg/kg bw (maximum recommended dose) and 1000 mg/kg bw. Negative controls received the vehicle alone. Dimethylnitrosamine and 2-acetylaminofluorene were used as positive controls. Hepatocytes were sampled from 3 animals per data point 2-4 hours and 12-16 hours after treatment. For each animal the net grain count (NNG), i.e. the number of grains present in the nucleus minus the mean number of grains in an equivalent area of cytoplasm, was determined in one hundred cells from 2 slides. The study was performed according to the relevant OECD guideline and in compliance with GLP.
Treatments did not produce clinical signs at any time. No increase in mean NNG value and in the incidence of cells in repair (with NNG >5) was observed in rats treated with ITX compared to the vehicle group. A distinct increase in NNG was observed in animals treated with the positive control substances.

EHDAB

The toxicological characteristics of EHDAB were evaluated by the Scientific Committee on Cosmetology in 1993, and re-evaluated in 1999 by the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP, 1999).
According to the SCCNFP, EHDAB shows low acute toxicity. EHDAB was tested in a

28-day oral toxicity study in rat at the doses of 100, 300 and 1000 mg/kg bw. Moderate or moderately severe testicular atrophy was observed at the highest dose; spleen pigmentation was observed both in males (at 1000 mg/kg) and females (at 300 and 1000 mg/kg); spleen weight was increased in females (at 1000 mg/kg) and liver weight both in males (at 1000 mg/kg) and in females (at 300 and 1000 mg/kg). Based on the evidence of pigmentation of the spleen in females, a NOAEL of 100 mg/kg bw/day was established. Similar results were reported from a NTP study (EPA/OTS; Doc #88-920000887).

No evidence of teratogenic potential was observed in a gavage study in rats. No evidence of genotoxicity was observed *in vitro* in the standard Ames or chromosomal aberration tests or in the micronucleus test in mouse bone marrow following administration of EHDAB by intraperitoneal injection. Tests for photo-mutagenicity in bacteria and in mammalian cells *in vitro*, only relevant for the evaluation of the safety of the use of EHDAB as sunscreen, also gave negative results.
Based on the toxicological profile, the SCCNFP concluded that a systemic exposure of 0.6 mg EHDAB/kg bw, as could result from the use of EHDAB as UV filter in cosmetic products, is of no toxicological concern.

Evaluation of toxicity data on ITX

In non-GLP studies ITX induced forward mutations in mammalian cells, and a borderline increase of revertant colonies in a bacterial reversion test. Equivocal results were provided by a chromosomal aberration test. *In vivo*, ITX was inactive in adequate genotoxicity tests in liver and bone marrow.

Structure-activity considerations indicate that ITX has no structural alerts for reactivity with DNA, but it shows some resemblance to the intercalating agents hycanthone and lucanthone, even though ITX lacks the polar group [-(2-diethylamino)ethylamino] which stabilizes the intercalating complex in the case of hycanthone and lucanthone. This structural feature could explain the weak positive response elicited by ITX toward the frameshift strain of *S.typhimurium* TA98, which is especially sentitive to intercalating agents.

Tests in mammalian cells *in vitro* gave contradictory results, showing a dose-related induction of small mutant clones in the mouse lymphoma *tk* system, which are mainly considered as expression of chromosomal rearrangements involving the *tk* locus, but no clear clastogenic effect in a similar dose range in a cytogenetic test in Chinese hamster cells. Therefore, there is an inconsistency in the results of tests in mammalian cells which cannot be resolved based on the limited information provided by the non-GLP *in vitro* studies performed. If confirmed, these data may indicate that ITX has the ability to induce gene mutations *in vitro* with no, or only minor effects at chromosomal level. However, it is noted that this is a relatively rare feature, displayed e.g. by some DNA base analogues. This feature is not anticipated for ITX based on chemical structure. Consequently the absence of clastogenicity observed in the micronucleus test can be taken as a more general evidence of lack of genotoxic potential *in vivo*.

It should also be borne in mind that the interpretation of the biological relevance of positive results in the mouse lymphoma system, without other supporting data, may not be straightforward in view of the possible involvement of mechanisms leading to loss of heterozygosity (e.g. miotic recombination, gene conversion) with unknown health significance (Henry *et al*., 1998). The inability of the mouse lymphoma assay to discriminate

non-carcinogens from carcinogens should also be noted (Kirkland *et al*., 2005).

For the purpose of hazard identification for human risk assessment, greater relevance is given to *in vivo* test results than to *in vitro* findings, provided that the studies address the same end-points. Based on present data, it cannot be established whether this condition is fulfilled for ITX. The *in vivo* micronucleus test gives some reassurance on the lack of systemic effects *in vivo*: even though there was no direct evidence that the test material reached the bone marrow, the results of this study show that oral administration of ITX at the maximum recommended dose did not produce detectable clastogenicity in a well-perfused tissue. However, as mentioned above, data from *in vitro* studies in mammalian cells could be interpreted as indicative of a mutagenic potential at the gene level independent of clastogenicity. The second *in vivo* assay, the UDS test in rat liver, does not add critical information for the evaluation of the genotoxicity of ITX. This study shows that *in vivo* ITX does not induce DNA damage repaired throught the nucleotide excision repair pathway, e.g. bulky DNA adducts, a conclusion which could be anticipated based on the lack of structural alerts for DNA reactivity.

In conclusion, the existing *in vivo* genotoxicity studies do not indicate a genotoxic potential for ITX.

In view of the lack of other toxicity data no further comment on the safety of ITX can be made.

Evaluation of toxicity data on EHDAB

The available data indicate that EHDAB is not genotoxic and not teratogenic. The NOAEL for general toxicity in 28-days oral studies in rats was 100 mg/kg. b.w. EHDAB exerted severe to moderate testicular toxicity at 1000 mg/kg.b.w. The mechanism for such effect is unknown, and the possibility exists that immature developing children be more sensitive than adults. However, with respect to the health risk related to the use EHDAB in inks applied to food packaging materials, the large margin of safety suggests that testicular toxicity is not a matter of concern.

CONCLUSIONS

ITX was tested with contradictory results in limited genotoxicity studies *in vitro*; however, clearly negative results were obtained in two adequate *in vivo* studies. In conclusion, the existing *in vivo* genotoxicity studies do not indicate a genotoxic potential for ITX. No other toxicity data on ITX are available.
The Panel noted that due to their high consumption of food per kg body weight, infants exclusively fed with infant formulae packed in cartons printed with UV-cured inks are potentially more exposed to ITX and EHDAB than other population groups.

In view of the lack of other toxicity data no further comment on the safety of ITX can be made.

EHDAB is not genotoxic and not teratogenic. The NOAEL for general toxicity in 4-week oral studies was 100 mg/kg b.w. A large (2500 or greater) margin of safety can be calculated for all exposure scenarios. It is concluded that the occurrence of EHDAB in food from its use in inks applied to food packaging materials is of no safety concern.

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