EUROPEAN COMMISSION
DIRECTORATE-GENERAL HEALTH AND CONSUMER PROTECTION
Directorate B - Scientific Opinions on Health Matters
Unit B2 - Management of Scientific Committees I
Scientific Committee on Toxicity, Ecotoxicity and the Environment

Brussels, 28/9/99
B2/JCD/csteeop/**cit28999**.D(99)

SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE)

Opinion on

the toxicological characteristics and risks of certain citrates and adipates used as a substitute for phthalates as plasticisers in certain soft PVC products

Opinion adopted at the 11th CSTEE plenary meeting

on the 28th of September 1999

1. Summary

The CSTEE has evaluated the toxicological characteristics and risks of certain citrates and adipates in order to examine whether such substances may be used as substitutes for phthalate plasticisers in PVC toys. In doing this, the CSTEE has applied the same general risk assessment principles used in its previous opinions on phthalates in PVC products that may be mouthed by children. The documentation made available to the CSTEE and the information found in the open literature on exposure and effects of the specified citrates and adipates, is too limited to determine whether they are safe to use as plasticisers in materials which may be mouthed by children.

2. Background

In its opinion of 24 April 1998 on 'Phthalates in toys', the CSTEE has recommended that *'before introducing other plasticisers into toys which children can put into their mouth, the risk of their use should be assessed by the same process which has been applied to the phthalates discussed above'*.

Recent announcements by toy manufacturers indicate that substitution of phthalates by other plasticisers will take place in the near future. In this contest citrates have been mentioned as possible promising candidates for such a substitution. Citric acid esters have been available since the 1940s for use as plasticisers in polymers such as polyvinyl chloride (PVC) and cellulose acetate. There are currently several manufacturers of citrate esters for use as plasticisers. Information from industry and national laboratories in Member States confirm the existing use of adipates as plasticisers in PVC toys. Also, there are a number of commercially available alternatives to PVC such as thermoplastic elastomers (styrenic block copolymers, polyolefin blends, elastomeric alloys), ethylene vinyl acetate and polyolefins (polyethylene, polypropylene) (CSTEE/98/17 - Add. 35).

The CSTEE has been presented with the following terms of reference on toxicological characteristics and risk to child health of certain citrates, and notably acetyltributyl citrate and diethylhexyl adipate, used as a substitute for phthalates as plasticisers in soft PVC toys and childcare articles:

1. What are the toxicological profiles of the substances under reference? What ranking of these substances can be made on the basis of their toxicological profiles?
2. How do the substances under reference compare with phthalates in terms of their toxicological profiles?
3. Does the CSTEE consider that the toxicological profiles of the substances under reference support their safe use as plasticisers in the products under consideration? Bearing in mind the potential for migration of these substances from the products under consideration, should limits for the migration of these substances from the products under consideration be set, and if so, which limits? In view of both the toxicological profile and the potential for migration of the substances under reference, does the CSTEE consider that the margins of safety for the use of these substances in products under consideration are adequate?
4. What are the issues on which additional information and/or research is required that may help answer the above questions?

The previously formed 'Phthalates Working Group' of the CSTEE has attempted to address these questions. It became readily apparent that there was less sound toxicological and exposure documentation on which to base qualified answers to the questions, this is especially the case for citrates and adipates other than acetyltributyl citrate and diethylhexyl adipate, respectively. Also, most of the information related to the citrates was not available in the open literature and has thus not undergone scientific peer review. In part due to confidentiality issues, the process of making the documentation available to the CSTEE has been slow. A considerable part of the toxicological data generated on the citrates is old and has not been developed applying modern test guidelines. The documentation on adipates has been gathered after searches in available databases and from a comprehensive evaluation report (BUA, 1996).

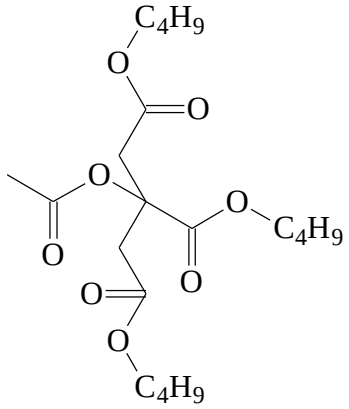
3. Citrates

3.1 O-Acetyltributyl citrate (ATBC)

3.1.1 Physicochemical characteristics

The following properties of ATBC have been identified in the literature (CSTEE/98/17 - Add 1; CSTEE/98/17 – Add.3; CSTEE/97/1-Add.116; CSTEE/97/1-Add.115; CSTEE/98/17 – Add.36):

CAS number: 77-90-7
EINECS number: 201-067-7
Molecular formula: $C_{20}H_{34}O_8$



Molecular weight: 402.5
Vapour pressure: 0.052 mm Hg (20°C)
Melting point: -80°C
Boiling point: 173 °C (1 mm Hg)
200 °C (4 mm Hg)
326 °C (160 mm Hg)
Decomposition temp: >220 °C
Solubility in water: 20 $mg.L^{-1}$
ethanol: Soluble
acetone: Soluble
DMSO: Soluble
toluene: Soluble

3.1.2 Migration from PVC products

Migration of plasticisers from food packaging materials into especially fatty food has been studied a lot. These studies have been performed with static methods (no mechanical treatment) which are known to give much lower results than the *in vivo* studies performed to mimic the mouthing/chewing of a small child.

The migration of ATBC from polyvinylidene chloride film into olive oil have been investigated and 2-30 $mg.dm^{-2}$ was observed. No time for the experiment was given (CSTEE/98/17 - Add. 1).

Medical grade PVC was blended with different plasticisers (about 30%) and moulded to films. These were extracted with different media and the following results were obtained (CSTEE/97/1-Add116):

Plasticiser	**DEHP**	**DEHA**	**ATBC**
Water extraction, %	0.7	1.5	1.2
Soapy water extraction, %	2.7	11.0	9.5
ASTM oil No. 3 extraction, %	11.4	34.7	10.9

The following specific migration (static, one-sided) of ATBC from PVDC film have been reported (CSTEE/98/17 - Add. 36):

Film type	**ATBC (%)**	**Simulant**	**Conditions**	**$mg.dm^{-1}$**
Household cling	4.9	Sunflower oil	10 days 40 °C	4.7
		3% acetic acid		2.8
Industrial cling	4.3	Sunflower oil		3.8
		3% acetic acid		1.5
Industrial non-cling	4.9	Sunflower oil		3.3
		3% acetic acid		2.2
Industrial non-cling	2.6	Olive oil	2 hr 70 °C	4.1
			10 days 40 °C	4.7

One of the producers of plasticisers have performed an extraction study to compare the migrations of ATBC and DINP from PVC (CSTEE/98/17 - Add. 33). The table below shows the loss of plasticiser to a saliva simulant at 60 °C during 24 hours in a static test.

Plasticiser concentration (%) in PVC	**ATBC (% loss)**	**DINP (% loss)**
40	0.8	3.4
65	2.0	6.1

The conditions during this study were rather extreme with 40 mm thick disks (about 50 mm diameter) and a high temperature under static conditions, thereby making it difficult to compare the outcome with results from other studies. The discs were also cleaned with an organic

solvent before the test. It may, however, be possible to compare the extraction efficiencies for the two investigated plasticisers, indicating a faster emission of the DINP compared to ATBC.

There are also several reports on migration of ATBC from plastic films during microwave treatment, but these are less relevant for the exposure of children and will not be reviewed here.

3.1.3 Exposure of children from PVC articles and other products

ATBC is used as a flavouring agent in food. From the used amounts (corrected for underestimation) and under the assumption that the whole amount ends up in the food supply of 10% of the consumers, the daily intake for these has been estimated to 0.02 microg/kg bw (JECFA, 1999).

No information has been found indicating the exposure of children from PVC articles or other products.

3.1.4 Toxicokinetics

ATBC is rapidly absorbed after oral administration in rats with a half-life of 1.0 hr (CSTEE/98/17 - Add. 46). Peak blood concentrations were observed 2-4 hours after administration. At least 67% of the dose is absorbed. The elimination from the blood was biphasic with half-lives of 3.4 hrs and 39 hrs, respectively. The long half-life of the second phase is presumably related to the incorporation of radiolabel into the carbon pool. There are no data on distribution of ATBC. The substance is primarily excreted into the urine (approx. 64%), excretion in faeces amounted to approx. 32% and expired air approx. 2%. ATBC is extensively metabolised, at least 9 metabolites, more polar than ABTC but less polar than citric acid, appear in the urine and at least 3 in faeces. Monobutyl citrate is the major urinary metabolite of ATBC. Theoretically ATBC could be hydrolysed to butanol, however, this has not been documented as a metabolite. There are no structural alerts in the ATBC molecule indicative of chemical reactivity.

3.1.5 Short-term effects

ATBC is virtually non-toxic after single gavage administration to rats and cats since doses of approximately 10 to 30 g/kg did not cause any systemic effects (CSTEE/98/17 - Add. 2).

3.1.6 Irritation

ATBC is not a skin irritant in rabbits, whereas it causes moderate eye irritation in rats (CSTEE/98/17 - Add. 4).

3.1.7 Sensitisation

ATBC did not appear to be a skin sensitiser when tested in the guinea pig maximisation test (CSTEE/98/17 - Adds. 6, 52). In contrast, acetyltriethyl citrate and triethyl citrate appeared to be strong sensitisers in this test. A sensitisation test with ATBC carried out in humans did not show any evidence for sensitising or irritating capacity (CSTEE/98/17 - Adds. 5, 54). Also, acetyltriethyl citrate and triethyl citrate gave a negative response in the human sensitisation test.

3.1.8 Repeated dose toxicity

In a 4-week range-finding feed study in rats, ATBC caused decreased body weights and changes in organ weights from feed concentrations of 2.5% onwards (corresponding to 2700 mg/kg bw/day) (CSTEE/98/17 - Add. 45). No effects were seen at lowest feed concentration of 1% ATBC in the diet (equal to 1000 mg/kg bw/day).

In a 90-day gavage study with male and female Wistar rats (according to OECD Guideline 408) haematological and biochemical changes were noted from 300 mg/kg bw/day onwards (CSTEE/98/17 - Add. 44). At 1000 mg/kg bw/day increased liver weights were observed in both sexes. No histopathological changes were seen. The NOAEL in this study is 100 mg/kg bw/day.

3.1.9 Genotoxicity

ATBC does not induce gene mutations in *Salmonella typhimurium* in the absence or presence of a metabolism system (CSTEE/98/17 - Adds. 10, 47). ATBC does not induce chromosomal aberrations in two studies with rat lymphocytes in the absence or presence of a metabolism system (CSTEE/98/17 - Adds. 48, 50). ATBC increased the mutant frequency of CHO cells (HGPRT-locus) at the highest concentration in the presence of a metabolism system in one experiment, this could not be repeated in a second experiment (CSTEE/98/17 - Add. 49). The compound could not be evaluated without a metabolism system due to severe cytotoxicity. ATBC caused a concentration-dependent increase in the mutant frequency of mouse lymphoma cells (TK-locus) in the presence of a metabolism system in two experiments, in one out of two experiments without a metabolism system increases were seen at the highest and lowest concentration (CSTEE/98/17 - Add. 36). ATBC did not cause unscheduled DNA synthesis (UDS) in rats treated by gavage with a single dose of 800 or 2000 mg/kg bw (CSTEE/98/17 - Add. 61). No other *in vivo* data are available with respect to genotoxicity testing of ATBC. Although there are suggestions of an *in vitro* genotoxic effect of ATBC, the negative UDS study indicates that the *in vivo* genotoxic potential of ATBC is low or absent.

3.1.10 Chronic toxicity/Carcinogenicity

In a two-year feeding carcinogenicity study in the Sherman rat (sex unspecified) (filed with the US FDA in 1950, CSTEE/98/17 - Add. 3), 20 rats per treatment group (40 controls) were given concentrations of 0, 200, 2000 and 20000 ppm ATBC in the diet (the highest dose corresponding to approximately 1000 mg/kg/day). Survival in the highest dose group was more than 50% percent. This study apparently did not reveal any significant toxicological findings related to ATBC exposure. However, the conduct and reporting of this study is not according to modern guidelines. It is not possible to properly evaluate the carcinogenic potential of ATBC from this study. It appears that ATBC is not a potent multi-site carcinogen, but the induction of a low incidence of a site-specific effect cannot be excluded.

3.1.11 Reproductive toxicity

A 2-generation reproduction study has been performed in Sprague-Dawley rats (according to OECD Guideline 416) with ATBC administered in the diet corresponding to doses of 0, 100, 300 and 1000 mg/kg bw/day (CSTEE/98/17 - Add. 36). Decreased body weights were seen from the mid-dose in F_1 male rats and at the high dose in F_0 male rats. No effects were seen in the pups. The NOAEL from this study is 100 mg/kg bw/day

There are no data available with respect to teratogenicity of ATBC.

3.1.12 Data gaps

There is limited knowledge on migration rates of ATBC from PVC products. From a single *in vitro* study it appears that the extraction loss of ATBC from PVC samples by saliva simulant extraction is approximately one third the rate of diisononyl phthalate (DINP). There is no information on exposure of children to ATBC from PVC products or other articles.

There is no evidence that ATBC is a skin sensitiser, although the structurally similar compounds acetyltriethyl citrate and triethyl citrate are strong sensitisers in guinea pigs. The underlying mechanism for these structural differences is not known. Since there was cross reactivity between acetyltriethyl citrate and triethyl citrate, it could be the triethyl tail which renders these citrates to be immunogenic.

There are deficiencies in the database with respect to genotoxicity of ATBC. There are some suggestions of *in vitro* genotoxicity, whereas one *in vivo* UDS study was negative. Preferably, an *in vivo* chromosomal mutation study should be carried out in order to have a more complete database for a conclusive evaluation of the genotoxic potential of ATBC.

A chronic toxicity/carcinogenicity study on ATBC in compliance with modern guidelines is not available. Since a well-conducted 2-generation reproduction study has been performed, this can be used as a substitute for a chronic toxicity study for identifying a No-Observable-Adverse-Effect-Level (NOAEL). Ideally, a chronic toxicity study on ATBC would be needed to substantiate that this is the proper NOAEL value. An in-depth evaluation of the carcinogenic potential of ATBC is not possible based on the data presented to the CSTEE.

Teratogenicity studies on ATBC are lacking, however, this is not seen as a data deficiency in the present exposure situation involving young children.

3.1.13 Critical effect and NOAEL

There are limited data on which to identify the critical effect and NOAEL properly. From the 2-generation reproduction toxicity study decreased body weight was identified as the critical effect giving a NOAEL of 100 mg/kg bw/day. A similar value was established from the 90-day repeated dose study.

3.1.14 Tolerable daily intake (TDI)

The Scientific Committee on Food (SCF) has placed ATBC on their list 7 of 1995, *Substances for which there were insufficient toxicological or technological data to enable the Committee to express an opinion*, and more specifically *Substances for which some toxicological data exist, but for which an ADI or a TDI could not be established* (CSTEE/98/17 - Add. 37). JECFA at its meeting in June 1999 evaluated the use of ATBC as a flavouring agent. According to the Procedure for the Safety Evaluation of Flavouring Agents (based on estimated intake) it was concluded that the intake does not exceed the exposure threshold of concern (1800 microgram/person/day) and there is no safety concern for its use as a flavouring agent (JECFA, 1999).

The CSTEE considers that it is not possible to do a proper risk assessment, especially because of the lack of exposure information. There also are deficiencies with respect to availability of effects information. A complete database is needed in order to evaluate the safety of a phthalate substitute for children's toys. Thus, it is not possible to set a TDI.

3.1.15 Intake doses from PVC articles

It is not possible to estimate intake doses in children mouthing PVC toys containing ATBC from the present database. Assuming, as indicated in section 3.1.2, ATBC is extracted more or less as effectively as the phthalates from PVC and the same concentrations are used in the polymers, a migration of up to 10 µg/10 cm^2/min could be expected from toys when chewed/mouthed by small children. If the released substance is fully hydrolysed this will give a total daily dose of about 200 microgram/kg butanol if a child weighing 5 kg chews the toys during 3 hours. Such a dose is without toxicological concern.

3.1.16 Other exposures

There are no specific data on ATBC exposure of children from other exposures. Except for the possible intake of ATBC as a flavouring agent, there are no specific data on exposure of children to this compound.

3.1.17 Margin of safety (MOS)

It is not possible to estimate the relationship between exposure levels to ATBC from mouthing soft PVC toys and its NOAEL, due to the data gaps.

3.1.18 Comparison with phthalates

The extraction of ATBC from PVC may be comparable to that of phthalate esters. As can be seen in section 3.1.2 there are indications of both somewhat higher and somewhat lower extractability of ATBC as compared to the phthalates, but the results indicate that they are at least of the same order of magnitude.

3.1.19 *Migration limits*

Migration limits for ATBC from PVC cannot be identified from the available data.

3.2 Other citrates

3.2.1 Triethyl citrate

CAS number: 77-93-0.

No information has been made available to the CSTEE on the extractability of triethyl citrate from PVC toys or the exposure of children from such toys.

The oral LD50 value for triethyl citrate in rats is approximately 7 g/kg (CSTEE/98/17 - Add. 2). The substance appears to be a strong sensitiser in the guinea pig maximisation test (CSTEE/98/17 - Add. 6). However, it did not show any evidence of sensitising capacity or skin irritation in humans (CSTEE/98/17 - Adds. 5, 54). Feeding triethyl citrate (highest dose

approx. 4 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs (CSTEE/98/17 - Add 2).

Data presented to the Scientific Committee for Food in 1990 showed that triethyl citrate is hydrolysed *in vivo* to citric acid and ethanol, compounds with well-defined, low toxic potential (CSTEE/98/17 - Add. 37/b). Triethyl citrate appeared to be hydrolysed at a slower rate with human serum compared to rat serum (CSTEE/98/17 - Add. 37/d).

If, as indicated for ATBC in section 3.1.2, triethylcitrate is extracted more or less as effectively as the phthalates from PVC and the same concentrations are used in the polymers, a migration of up to 10 $\mu g/10cm^2$/min could be expected from toys when chewed/mouthed by small children. If the released substance is fully hydrolysed this will give a total daily dose of about than 120 microgram/kg ethanol if a child weighing 5 kg chews the toys during 3 hours. Such a dose is without toxicological concern.

No other toxicological data on triethyl citrate have been available to the CSTEE, although the Scientific Committee for Food refers to an older, inadequate long-term study in the rat (CSTEE/98/17 - Add. 37/b).

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) established in 1979 a temporary ADI of 10 mg/kg bw. This was changed in 1984 to an ADI of 20 mg/kg bw. The Scientific Committee for Food agreed in 1981 and 1990, respectively, to these values (CSTEE/98/17 - Add. 37/b). The Scientific Committee for Food has placed triethyl citrate on its positive list, List 1 of 1995, *Substances, e.g. food additives, for which an ADI, a temporary ADI (t-ADI), a MTDI, a PMTDI, a PTWI or the classification "acceptable" has been established by this Committee or by JECFA* (CSTEE/98/17 - Add. 37). JECFA at its meeting in June 1999 evaluated the use of triethyl citrate as a flavouring agent according to the Procedure for the Safety Evaluation of Flavouring Agents. Based on estimated intake for Europeans of 3400 microgram/person/day, it was concluded that the intake exceeds the exposure threshold of concern (1800 microgram/person/day), but that there is no safety concern for its use as a flavouring agent (JECFA, 1999).

Triethyl citrate is a strong sensitiser in guinea pigs using the maximisation test in which the compound was injected adjuvant, although no sensitising capacity for humans was apparent from a repeated insult patch test. Further, it failed to induce irritation in human skin. Thus, triethyl citrate will not readily lead to sensitisation when in contact with normal human skin. However, it cannot be ruled out that it will induce sensitisation when in contact with human skin or mucous membranes that is damaged or affected in such a way that inflammatory responses are present.

3.2.2 Acetyltriethyl citrate

CAS number: 77-89-4.

No information has been made available to the CSTEE on the extractability of acetyltriethyl citrate from PVC toys or the exposure of children from such toys.

The oral LD50 value for acetyltriethyl citrate in rats is approximately 7 g/kg (CSTEE/98/17 - Add. 2). The substance causes slight to moderate eye irritation in rabbits (CSTEE/98/17 - Add. 4). Acetyltriethyl citrate appears to be a strong sensitiser in the guinea pig maximisation test (CSTEE/98/17 - Add 6). However, it did not show any evidence of sensitising capacity or skin irritation in humans (CSTEE/98/17 - Adds. 5, 54). Feeding the substance (highest dose approx. 4 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs.

No other toxicological data on acetyltriethyl citrate have been available to the CSTEE.

Acetyltriethyl citrate is currently on the Scientific Committee for Food List 8 of 1995, *Substances for which there were insufficient toxicological or technological data to enable the Committee to express an opinion*, and more specifically *Substances for which no or only scanty and inadequate data were available* (CSTEE/98/17 - Add. 37).

Acetyltriethyl citrate is a strong sensitiser in guinea pigs using the maximisation test in which the compound is injected in adjuvant, although no sensitising capacity for humans was apparent from a repeated insult patch test. Further, it failed to induce irritation in human skin. Thus, acetyltriethyl citrate will not readily lead to sensitisation when in contact with normal human skin. However, it cannot be ruled out that it will induce sensitisation when in contact with human skin that is damaged and affected in such a way that inflammatory responses are present.

3.2.3 Tributyl citrate

CAS number: 77-94-1.

No information has been made available to the CSTEE on the extractability of tributyl citrate from PVC toys or the exposure of children from such toys.

Tributyl citrate is virtually non-toxic after single gavage administration to rats and cats in that doses of approximately 10 to 30 g/kg did not cause any systemic effects (CSTEE/98/17 - Add. 2). Feeding the substance (highest dose approx. 20 g/kg/d) mixed in the diet to rats for 6-8 weeks apparently did not result in deleterious effects on growth and nutrition, blood parameters or gross or histological appearance of the thoracic and abdominal organs.

No other toxicological data on tributyl citrate have been available to the CSTEE.

The Scientific Committee for Food has placed tributyl citrate in its List 6B of 1995, *Substances for which there exist suspicions about their toxicity and for which data are lacking or are insufficient. (The allocation of substances to this list is mainly based upon similarity of structure with that of chemical substances already evaluated or known to have functional groups that indicate carcinogenic or other severe toxic properties)*, and more specifically *Section 6B: Substances suspected to have toxic properties (other than carcinogenic). Restrictions may be indicated* (CSTEE/98/17 - Add. 37).

3.2.4 *Evaluation*

Triethyl citrate is a potential skin sensitiser for humans. There is no relevant exposure information on the substance and the toxicological database is limited. Thus, it is not possible to perform a proper risk assessment of exposure to children of triethyl citrate from PVC toys.

Acetyltriethyl citrate is a potential skin sensitiser for humans. There is no relevant exposure information on the substance and the toxicological database is extremely limited. Thus, it is not possible to perform a proper risk assessment of exposure to children of acetyltriethyl citrate from PVC toys.

Tributyl citrate has an extremely limited toxicological database and there is no relevant exposure information on the substance. Thus, it is not possible to perform a proper risk assessment of exposure to children of tributyl citrate from PVC toys.

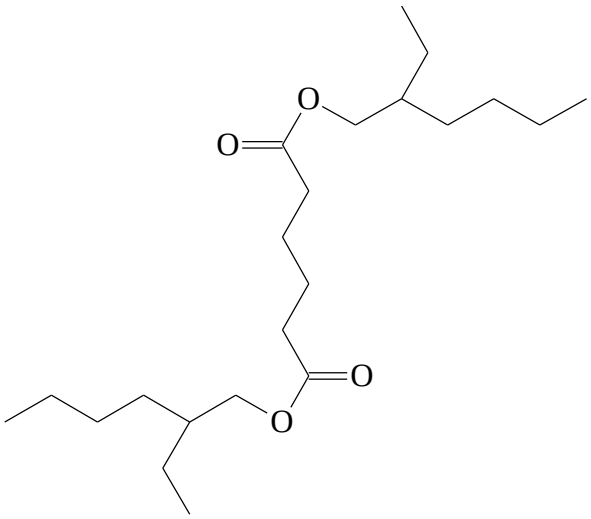
4 Adipates

4.1 Diethylhexyl adipate (DEHA)

4.1.1 Physicochemical characteristics

The following properties of DEHA have been identified in the literature (IUCLID 1996):

CAS number: 103-23-1
EINECS number: 203-090-1
Molecular formula: $C_{22}H_{42}O_4$



Molecular weight: 370.58
Vapour pressure: 0.021 hPa (100 °C)
Melting point: -76 °C
Boiling point: 210-218°C (7 hPa)
$\log P_{ow}$: 8.114
Solubility in water: <100 $mg.L^{-1}$ (20 °C)

4.1.2 Migration from PVC products

The migration of DEHA from PVC film into different foods has been investigated and it is obvious that high lipid content in the food increase the migration (Harrison, 1988). In the same report a maximum dietary intake of DEHA due to this contamination was calculated to 16 $mg.kg^{-1}.day^{-1}$.

In another study the intake of DEHA in the UK was estimated from the concentration of 2-ethylhexanoic acid in urine samples from 112 adults. The results showed a skewed distribution with a median value of 2.7 $mg.day^{-1}$ with a maximum of 8.2 $mg.day^{-1}$ (Loftus et al., 1994).

Urine sampled over 24 hours by approximately 50 male participants from France, Germany and the Netherlands was also analysed for the DEHA metabolite. The median exposure for these three countries was estimated to 1.04, 0.80 and 0.86 mg DEHA/day, respectively (Woollen, 1998).

The content of plasticisers in baby food have been investigated in Denmark (Breidendahl and Petersen, 1998). Of 11 investigated "ready to use" infant formulae DEHA was found in 2 (0.02 and 0.05 mg DEHA/kg), while DEHA was not found in any of the 11 studied baby foods. The content of plasticisers in 21 total diet samples for adults were also measured in this study and the results are shown in the following table:

	Plasticiser amount in total diet (mg/10 mJ)			
	DBP	**BBP**	**DEHA**	**DEHP**
Range	0.13-0.29	0.02-0.03	0.20-0.21	0.19-0.30

In a Danish survey plastic film on the market were tested for DEHA migrations to olive oil (10 days at 40°C). Of the 49 investigated samples 42 exceeded the action limit set at 4 $mg.cm^{-2}$ (Breidendahl and Petersen, 1998).

Models are developed for the prediction of migration of DEHA from plasticised PVC film into different food types and the result is compared with earlier measured data (Mercer et al., 1990). The measured migration varied between 0.6 and 19 $mg.dm^{-2}$ and the result does not seem to change dramatically between 1 and 7 days exposure.

The studies of migration of DEHA into foodstuffs have been published (BUA, 1996) and the maximum value is observed in Brie cheese. After 5 days at 5°C up to 195 $mg.dm^{-1}$ had been transferred from the PVC film containing 17.2% DEHA.

No specific documentation has been found related to the migration of DEHA from PVC toys using salivary simulants. The tests of migration of DEHA into food from packaging materials have been carried out without any mechanical stress (static tests), therefore these results are difficult to extrapolate to the extraction in the mouth of a child.

4.1.3 Exposure of children from PVC articles

No information has been found describing the exposure of children to DEHA from PVC articles.

4.1.4 Toxicokinetics

DEHA is rapidly and completely absorbed from the gastrointestinal tract of experimental animals. In rats, there is evidence for cleavage of the parent compound and subsequent absorption of the monoester and the acid, whereas in cynomolgus monkey also unchanged DEHA is absorbed. DEHA is distributed to a number of tissues with maximum levels reached after 6-12 hours. Liver, fat, kidney and adrenals had relatively high levels of DEHA-associated radiolabel, whereas large amounts of radioactivity were found in the gastrointestinal tract (BUA, 1996).

After oral administration, DEHA is hydrolysed in the gastrointestinal tract to 2-ethylhexanol, mono(2-ethylhexyl)adipate and adipic acid. A half-life of 6 minutes for metabolism of DEHA has been determined in rat small intestine mucus membrane homogenates. The main urinary DEHA metabolite in rats is by far adipic acid (80-90% of administered oral dose). Other major metabolites are 2-ethylhexanoic acid glucuronide and 2-ethyl-1,6-hexanedoic acid. In the monkey the glucuronide of mono(2-ethylhexyl)adipate and traces of unchanged DEHA were found in the urine (BUA, 1996).

In humans given deuterium-labelled DEHA, 2-ethylhexanoic acid was the only metabolite that could be determined in the plasma. It had an elimination half-life of 1.65 hours. In urine, the following metabolites were identified (percentage fraction of administered radioactivity): 2-ethylhexanoic acid (8,6%), 2-ethyl-5-hydroxyhexanoic acid (2.6%), 2-ethyl-1,6-hexanedioic acid (0.7%), 2-ethyl-5-ketohexanoic acid (0.2%), and 2-ethylhexanol (0.1%). The half-life for elimination of all metabolites excreted with the urine averaged 1.5 hours, none of the metabolites could be detected after 36 hours (BUA, 1996).

DEHA is rapidly eliminated, with most of the ^{14}C-radioactivity appearing in the urine after oral administration of rats, mice and cynomolgus monkeys (rats: 34-78% of the dose after 24 hours; mice: 75-92%; monkeys: 47-57%). In rats, the total radioactivity in the body after 96 hours was approx. 0.5%. Some of the biliary (approx. 3% in rats) secreted radioactivity flows into the enterohepatic circulation. Passage of DEHA through the placenta of pregnant mice has been described (BUA, 1996).

4.1.5 Short-term effects

DEHA has very low acute toxicity, the following LD50 values have been reported: Rat (oral) 7,392-45,000 mg/kg bw; mouse (oral) 15,000-24,600 mg/kg bw; rabbit (dermal) 8,410-15,100. The symptoms of intoxication in the rat following oral administration were co-ordination disorders (BUA, 1996).

4.1.6 Irritation

DEHA has been reported to be non-irritating or slightly irritating to the skin of rabbits in some studies. Also, non-irritation or slight eye irritation have been reported in some studies (BUA, 1996; IUCLID, 1999).

4.1.7 Sensitisation

A Draize test failed to produce symptoms of a sensitising potential of DEHA (BUA, 1996).

4.1.8 Repeated dose toxicity

A number of studies have shown DEHA to induce changes indicative of peroxisome proliferation in the liver of rats when the compound is orally administered at dosages generally higher than 1,000 mg/kg bw for 5 to 30 days. Dose dependent changes included increases in relative liver weight, reduction in serum triglyceride and cholesterol levels, increase in hepatic catalase and carnitine acyl transferase activity, as well as biochemical and morphological evidence of peroxisome proliferation. The effects were more pronounced in male rats compared to females. DEHA also acts as a peroxisome proliferator in mice. The peroxisome proliferation appears to be caused by metabolites, rather than the parent compound, with 2-ethylhexanoic acid being the most active metabolite. The peroxisomal effects of DEHA are moderate compared to those of DEHP, which shows a NOAEL for peroxisome proliferation at 5 mg/kg bw/day (RIVM, 1992). There is a marked species difference for the peroxisomal effects. *In vitro* studies with hepatocytes of rats, guinea pigs and marmosets show only in rat hepatocytes a clear effect (BUA, 1996).

There are no adequately performed studies which allow a precise determination of a NOAEL for DEHA from subchronic or chronic studies. An oral rat 90-day study from 1951 quotes a NOAEL of 610 mg/kg bw/day. In one 21-day feeding study in female F344 rats, 122 mg/kg bw/day was cited as the lowest dose which significantly increased peroxisome proliferation. A recent 2-week feeding study in Wistar rats showed a NOAEL of 200 mg/kg bw/day for induction of peroxisomal associated enzymes (BUA, 1996). In a 21-day feeding study in mice, a NOAEL of 325 mg/kg bw/day for peroxisomal proliferation was identified (IUCLID, 1999). The Scientific Committee for Food has assigned a NOAEL for DEHA in the rat, as measured by biochemical parameters and electronmicroscopic analysis of peroxisome proliferation, at around 100 mg/kg bw/day (CSTEE/98/17 - Add. 37/g).

4.1.9 Genotoxicity

DEHA has not induced point mutations in *Salmonella typhimurium* or mouse lymphoma cells, sister chromatide exchanges in primary rat hepatocytes or Chinese hamster ovary cells, nor unscheduled DNA synthesis in primary rat hepatocytes. Further, DEHA did not cause chromosomal aberrations or micronuclei in primary rat hepatocytes. In one test on Chinese hamster ovary cells, an increased rate of chromosomal aberrations was seen in the absence of a metabolic activation system, however, this study did not address cytotoxicity. DEHA has not induced micronuclei in mouse bone marrow cells or sex-linked recessive lethals in *Drosophila melanogaster*. In a dominant-lethal test in mice using intraperitoneal administration, a slight positive effect was seen. At the same time there was a reduction in the fertility index (not seen in oral studies), suggesting cytotoxicity rather than mutagenicity being the underly-

ing cause for the dominant lethality (BUA, 1996). DEHA did not induce cell transformation in Balb-3TR mouse embryo cell cultures (IUCLID, 1999). In an overall assessment of the test results, the CSTEE arrives at the conclusion that DEHA does not have a genotoxic potential.

4.1.10 Carcinogenicity

B6C3F1 mice fed 0, 12000 or 25000 ppm DEHA corresponding to doses of 1,800 and 3,750 mg/kg bw/day (EPA) for 103 weeks showed a dose-dependent incidence of hepatocellular tumours (adenomas and carcinomas combined) in both sexes. The number of females with hepatocellular carcinomas only was also significantly higher in both treatment groups. The male animals of the high dosage group also showed a significantly higher incidence of hepatocellular adenomas only (BUA, 1996).

F344 rats fed 0, 12000 or 25000 ppm DEHA corresponding to doses of 600 and 1,250 mg/kg bw/day (EPA) for 103 weeks did not show evidence of a substance-related carcinogenic effect (BUA, 1996).

In a study designed to explain the underlying species differences in hepatocarcinogenicity of DEHA, the substance showed sustained replicative DNA synthesis at dose levels (2.5% feed concentration) in female mice which were not effective in female rats (4.0% feed concentration). On the other hand, the magnitude of induction of peroxisome proliferation was similar in both species (Lake et al., 1997).

A covalent DNA-binding study in mouse liver and a cell transformation test in BALB/3T3 mouse cells were negative. On the other hand, increased levels of 8-OH-guanine adducts in rat liver DNA have been found after DEHA administration, indicative of the formation of reactive oxygen species (Takagi et al., 1990).

The proposed mechanisms whereby peroxisome proliferators induce liver tumours in rodents include oxidative stress, increased hepatocellular proliferation and/or preferential growth of preneoplastic lesions (IARC, 1995). The available evidence indicates that peroxisome proliferation in mouse and rat liver is mediated by activation of peroxisome proliferator-activated receptors (PPARs), which are members of the steroid hormone receptor superfamily. PPAR expression in human liver is much lower than that observed in mice (Palmer et al., 1998). The CSTEE considers the hepatocarcinogenic response of DEHA in mice to be a dose-thresholded phenomenon. Because of this, and the differences in sensitivity between humans and rodents towards peroxisome proliferators, exposures of children to DEHA orders of magnitude below those doses which induce liver tumours in mice, do not raise any concern.

4.1.11 Reproductive toxicity

In a developmental toxicity study in pregnant Wistar rats fed 0, 300, 1800 or 12000 ppm DEHA, stated by BUA (1996) and IUCLID (1999) to correspond to doses of 0, 28, 170 or 1080, or by the Scientific Committee for Food (CSTEE/98/17 - Add. 37/g) to doses of 0, 30, 110 or 720 mg/kg bw/day (The CSTEE notes that the Scientific Committee for Food may have miscalculated the low dose). The highest dose led to slight reductions in maternal body weight gain and food consumption. In the foetuses at the high dose, reduced ossification and kinked or dilated ureters were found. There was also a slightly significant increase of ureter kinking at the middle dose. The Scientific Committee for Food has in 1994 established a NOAEL for foetotoxicity at 30 mg/kg bw/day (CSTEE/98/17 - Add. 37/g).

In a companion one-generation reproduction toxicity study, Wistar rats were fed with DEHA corresponding to the same doses in the developmental toxicity study. No effects were seen on male or female fertility. The parental generation was fed continuously throughout the study for approx. 18-19 weeks of exposure. At the highest dose of 1080/720 mg/kg bw/day, there was a reduction in the body weight gain of the dams during gestation, an increase in liver weight in both male and female parents, and reductions in offspring weight gain, total litter weight and litter size. From this study a NOAEL of 170 (BUA, 1996) or 110 (SCF: CSTEE/98/17 - Add. 37/g) mg/kg bw/day for both maternal and foetal toxicity can be identified.

A drinking water study where female Long-Evans rats were exposed to di(2-ethylhexyl)-phthalate (DEHP) from day 1 of pregnancy to day 21 after delivery, identified severe histological damage to the testes of the offspring at 32.5 µl DEHP/L (Arcadi et al., 1998). Because of the similarities in chemical structure and metabolism between DEHA and DEHP, DEHA could potentially have a comparable profile to DEHP with respect to testicular toxicity in very young animals (DEHP NOAEL 3.7 mg/kg bw/day; Poon et al., 1997). The CSTEE considers that the one-generation reproduction study may not properly address this issue.

4.1.12 Data gaps

Specific data on the migration of DEHA from PVC products with salivary simulants are lacking. There is limited information on additional exposures of children to DEHA.

Studies to reveal a possible testicular toxic potential of DEHA after foetal and early postnatal exposure are lacking.

4.1.13 Critical effect and NOAEL

DEHA has a toxicological profile similar to DEHP, but is considerably less potent. The most sensitive effect identified so far is foetotoxicity. The lowest NOAEL for this effect is in the order of 30 mg/kg bw/day .

4.1.14 Tolerable daily intake (TDI)

DEHA is on the Scientific Committee for Food List 2 of 1995, *Substances for which the committee was able to express an opinion*, and more specifically *Substances for which a TDI or a t-TDI has been established by this Committee*. Using the NOAEL of 30 mg/kg bw/day for foetotoxicity and an uncertainty factor of 100, the Scientific Committee for Food has established a TDI for DEHA of 0.3 mg/kg bw (CSTEE/98/17 - Add. 37/g).

Given the specific exposure circumstances under consideration and that the structural analogue DEHP has testicular toxicity after pre-/perinatal exposure as its critical effect, the CSTEE considers it premature to establish a TDI for DEHA without a better database to judge any testicular toxic potential of this substance.

4.1.15 Intake doses from PVC articles

It is not possible to assign intake doses of DEHA from children mouthing PVC toys containing this plasticiser.

4.1.16 Other exposures

Mean DEHA exposures to the general population have been measured to be between 0.8 and 2.7 mg/day in 4 EU countries. The main source is assumed to be food packaging materials.

4.1.17 Margin of safety (MOS)

The relationship between exposure levels to DEHA and its NOAEL cannot be estimated because of lack of specific exposure information.

4.1.18 Comparison with phthalates

Three times as much DEHA compared to DEHP is extracted from PVC film into an oil (CSTEE/97/1-Add. 116, see 3.1.2). There are no data on the specific migration of DEHA into salivary simulants which allows a comparison with DEHP. The toxicological profile of DEHA is somewhat similar to, but less potent than DEHP, at least with respect to peroxisome proliferation.

4.1.19 Migration limits

Migration limits for DEHA in soft PVC articles cannot be set.

4.2 Other adipates

The CSTEE has not been supplied with documentation on dicapryl, diisobutyl, diisodecyl or dinonyl adipate and has not found information in the open literature on the migration, exposure and toxicology of these substances.

5 Conclusion

5.1 Terms of reference 1

There are important limitations regarding the toxicological database on O-acetyltributyl citrate (ATBC). The substance has not been studied for chronic toxicity and carcinogenicity according to modern test guidelines. There also are deficiencies in the data base with respect to genotoxicity. Thus at present, the CSTEE cannot evaluate the toxicological profile of this substance on all important endpoints.

Due to its sensitising potential, the CSTEE does not consider triethyl citrate to be a suitable substitute for phthalates as plasticisers in children' toys.

Due to its sensitising potential the CSTEE does not consider acetyltriethyl citrate to be a suitable substitute for phthalates as plasticisers in children' toys. In addition, the database on acetyltriethyl citrate is extremely limited with respect to assessment of additional toxicological endpoints.

The database on tributyl citrate is extremely limited with respect to toxicological endpoints, thus the CSTEE cannot properly evaluate the toxicological profile of this substance.

From the available toxicological data on DEHA, this substance appears to have low toxicity after long-term administration. It induces liver tumours in mice after high doses, but this effect is not considered to be of concern in the present situation given the underlying mechanism of carcinogenicity in mice and the large difference between maximum theoretical exposure doses in children and doses which are carcinogenic in mice. However, a proper assessment of a potential testicular toxic effect of DEHA after foetal/perinatal exposure cannot be performed from the existing data base.

No data have been available to the CSTEE regarding exposure and effects of the other adipates under consideration, therefore a risk assessment is impossible.

5.2 Terms of reference 2

Due to the limitations in the database for ATBC, it is not possible to compare this substance with the phthalates.

DEHA is less potent than DEHP in causing hepatic peroxisome proliferation. However, data are lacking allowing for a comparison of these structural analogues with respect to the critical effect of DEHP, namely testicular toxicity.

5.3 Terms of reference 3

Because of the important data gaps with respect to toxicology, and the dearth of specific migration data, the CSTEE considers that it is at present not possible to support the use of the reference citrates and adipates as plasticisers in the products under consideration. In principle, limits for the migration of these substances from such products could be set given that complete databases were available and that no unacceptable effects were revealed. However, such

limits cannot at present be set. It is not possible to examine the relationship between exposure levels and no effect levels, since data on which to make such comparisons are not sufficient.

5.4 Terms of reference 4

The CSTEE considers that databases on exposure and effects of the citrates and adipates must be comparable in breadth and quality to that of the phthalates, in order to properly evaluate their suitability as substitutes for phthalates as plasticisers in children's toys. Due to the sensitising potential of acetyltriethyl citrate and ethyl citrate, the CSTEE considers that these substances are not candidate alternatives to the phthalates.

5.5 Other considerations

In assessing the toxicological characteristics and risks of certain citrates and adipates which may be used as potential substitutes for phthalates as plasticisers in PVC toys, the CSTEE has applied generally accepted principles of risk assessment. Such assessments are able to assign safe levels of exposure to nongenotoxic chemicals from identification of no-effect levels in toxicological long-term studies and incorporation of appropriate uncertainty factors.

A very important and overall premise for risk assessment of substitution materials, is that the exposure and toxicological databases on the substitutes must be of sufficient quality and cover all the critical endpoints, so that a proper scientific assessment can be carried out. In the case of the citrates and adipates that the CSTEE have considered as substitutes to the phthalates, there are important data gaps with respect to both exposure and toxic effect information.

The CSTEE has given opinions on phthalates, citrates and adipates used as plasticisers in PVC products, since these are, or may be assumed to be, readily extractable from such products when children are mouthing PVC toys. The CSTEE has not evaluated the safety of PVC *per se* in children's toys, since this was not included in the terms of reference to the Committee. However, high-molecular weight polyvinyl chloride is a polymeric material which in itself is not bioavailable when toys are being mouthed by children and thereby non-toxic, unless the PVC product contains additives or residues at levels above those which are estimated to be safe.

The terms of reference given to the CSTEE relate to PVC products, and not to other materials which are or may be used in toys mouthed by children. The CSTEE is aware that there are a number of commercially available alternatives to PVC. Any assessment of the potential risks to children that may result from the use of alternatives to PVC in toys, should follow the same process of risk assessment that the CSTEE has used for plasticisers in PVC. Such a risk assessment must be based on the magnitude, frequency and duration of exposure to those substances which may be extracted from the alternative materials and on data from toxicological tests with such substances on critical endpoints.

6. Statement on the toxicological evaluation

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible.

CSTEE's opinions include evaluations of experiments using laboratory animals; such tests should be conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available should such tests be evaluated and the data accepted, in order to meet the fundamental requirements of protection of consumer health.

7. References

Arcadi FA, Costa C, Imperatore C, Marchese A, Rapisarda A, Salemi M, Trimarchi GR, Costa G. Oral toxicity of bis(2-ethylhexyl)phthalate during pregnancy and suckling in the Long-Evans rat. Fd Chem Toxicol 1998; 36: 963-970.

Breindahl T and Petersen JH. Plasticisers in infant formulae, baby food and total diet samples. Odense Landsdelslaboratorium, Intern rapport IFE 1998:4, 46 p, in Danish.

BUA. German Chemical Society Advisory Committee on Existing Chemicals of Environmental Relevance. Di-(2-ethylhexyl)adipate. BUA Report 196. S. Hirzel Wissenshaftliche Verlagsgesellschaft, Stuttgart, 1996.

Harrison N. Migration of plasticizers from cling-film. Fd Add Contam 1988; 5: 493-499.

IARC. Peroxisome Proliferation and its role in Carcinogenesis. International Agency for Research on Cancer. IARC Technical Report No. 24, Lyon, 1995.

IUCLID. Bis(2-ethylhexyl)adipate IUCLID Data Sheet. European Chemicals Bureau – Existing Chemicals, Ispra, 1999.

JECFA Report of June 1999 meeting, in press

Lake BG, Price RJ, Cunninghame ME, Walters DG. Comparison of the effects of di-(2-ethylhexyl)adipate on hepatic peroxisome proliferation and cell replication in the rat and mouse. Toxicology 1997; 123: 217-226.

Loftus NJ, Woollen BH, Steel GT, Wilks MF, Castle L. An assessment of the dietary uptake of di-2-(ethylhexyl)adipate (DEHA) in a limited population study. Fd Chem Toxicol 1994; 32: 1-5.

Mercer A, Castle L, Comyn J, Gilbert J. Evaluation of a predictive mathematical model of di-(2-ethylhexyl)adipate plasticizer migration from PVC film into foods. Fd Add Contam 1990; 7: 497-507.

Palmer CN, Hsu MH, Griffin KJ, Raucy JL, Johnson EF. Peroxisome proliferator activated receptor-alpha expression in human liver. Mol Pharmacol 1998; 53: 14-22.

Petersen JH, Breindahl T. Specific migration of di-(2-ethylhexyl)adipate (DEHA) from plasticized PVC film: results from an enforcement campaign. Fd Add Contam 1998; 15: 600-608.

Poon R., Lecavalier P., Mueller R., Valli VE, Procter BG, Chu I (1997) Subchronic oral toxicity of di-n-octyl phthalate and di(2-ethylhexyl)phthalate in the rat. Fd Chem Toxicol. 35, 225-239.

RIVM (1992). Jansen EHJM et al. Toxicological investigation of di(2-diethylhexyl)phthalate in rats. The determination of a no-effect level. Report No. 618902 007. Dated December 1992.

Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa 7: Significant increase of 8-hyddroxydeoxyguanosine in liver DNA of rats following short-term exposure to the peroxisome proliferators di(2-ethylhexyl)phthalate and di(2-ethylhexyl)adipate. Jpn J Cancer Res 1990; 81: 213-215.

Woollen BH. Survey into the dietary intake of di-2(ethylhexyl)adipate in Member States of the European Union. Report No: CTL/R/1372, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 1998, 32p.