EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

2,4-Diaminophenoxyethanol and its salts

COLIPA N° A42

Adopted by the SCCP
during the 7th plenary meeting of 28 March 2006

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1. BACKGROUND

Submission I for 2,4-Diaminophenoxyethanol was submitted in January 1988 by COLIPA[1, 2].

The Scientific Committee on Cosmetology (SCC) adopted at its 54th plenary meeting on 10 December 1993 the opinion on 2,4-Diaminophenoxyethanol dihydrochloride (CAS 66422-95-5) with the conclusion:

"*The SCC does not consider the use of 2,4-diaminophenoxyethanol in hair dyes to be linked to any particular toxic risk for consumers.*"

The substance is currently regulated by the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 36 on the List of substances provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission II for 2,4-Diaminophenoxyethanol was submitted by COLIPA in July 2005. According to this submission 2,4-Diaminophenoxyethanol is an ingredient of oxidative hair colouring products. It is used at a maximum final (on-head) concentration of 2.0%, after mixing the hair dye formulation with a hydrogen peroxide preparation typically in 1:1 proportions.

Submission II presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 2,4-Diaminophenoxyethanol in the form of its either dihydrochloride or sulphate salt safe for use as an oxidative hair dye with a concentration on-head of maximum 2.0 % taken into account the scientific data provided?*

2. *Does the SCCP recommend any further restrictions with regard to the use 2,4-Diaminophenoxyethanol in the form of its either dihydrochloride or sulphate salt in any oxidative hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

[2] According to records of COLIPA

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

2,4-diaminophenoxy ethanol HCl (INCI)
2,4-diaminophenoxyethanol sulphate (INCI)

3.1.1.2. Chemical names

2-(2´,4´-diaminophenoxy)ethanol
1-β-hydroxyethyloxy-2-4-diamino-benzene, dihydrochloride
2-(2´,4´-diaminophenoxy)ethanol dihydrochloride
1-β-Hydroxyethyloxy-2,4-diaminobenzene, sulphate

3.1.1.3. Trade names and abbreviations

IMEXINE OAJ (dihydrochloride)
COLIPA n^{o} A042

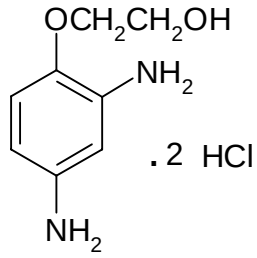
3.1.1.4. CAS / EINECS number

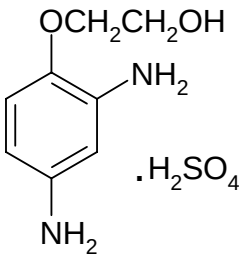
Free Base
CAS : 70643-19-5
EINECS : /

Dihydrochloride
CAS : 66422-95-5
EINECS : 266-357-1

Sulphate
CAS : 70643-20-8
EINECS : 274-713-2

3.1.1.5. Structural formula





3.1.1.6. Empirical formula

Free base
Formula: $C_8H_{12}N_2O_2$

Dihydrochloride
Formula: $C_8H_{12}N_2O_2$, 2HCl

Sulphate
Formula: $C_8H_{12}N_2O_2$, H_2O_4S

3.1.2. Physical form

Light grey to light pink powder (dihydrochloride)
White powder (sulphate)

3.1.3. Molecular weight

Molecular weight: 241.12 (dihydrochloride)
266.27 (sulphate)

3.1.4. Purity, composition and substance codes

Purity and impurities in various batches of 2,4-diaminophenoxyethanol HCl:

Description	**Batch n°**		
	0120022	**0101297**	**Op.118**
Chemical identification and chemical characterisation	IR, NMR, MS and elemental analysis		
UV spectrum	Comparable UV spectra		
HPLC profile (area %, without response factor)	>99.5		
Alkalinity titre by potentiometry ($HCLO_4$), % w/w	98.7	99.6	99.4
Melting point	242.5°C		

Description	Batch n°		
	0120022	**0101297**	**Op.118**
Impurity A: m-phenylenediamine	<100 ppm		
Impurity B: 2,4-diaminoanisole	Not detected Detection limit: 100 ppm		
Isopropanol	< 100 ppm		
Ash content, % w/w	0.1		
Water content, % w/w	0.02		
Loss on drying, % w/w	<0.1		
Metal content, ppm	As, Sb, and Hg: <5, Cd: <10, Pb: <20		

3.1.5. Impurities / accompanying contaminants

See 3.1.4.

3.1.6. Solubility

Dihydrochloride
Water: 425 ± 7 g/l (20 °C ± 0.5 °C) (EEC method A6)
Ethanol: ≤ 1 g/ 100 ml
DMSO: ≥ 10 g/100 ml

3.1.7. Partition coefficient (Log P_{ow})

Dihydrochloride
Log P_{ow}: 0.99 at 20°C±0.5°C (EEC method A8)

3.1.8. Additional physical and chemical specifications

Dihydrochloride

Melting point: 242.5 °C
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /

3.1.9. Stability

2,4-Diaminophenoxyethanol HCl (batch No. 0120022) as 0.1 and 200 mg/ml in aqueous solution was shown to be stable up to 6 h at room temperatures, 9 days at + 4 °C, protected from light and under inert gas atmosphere.

2,4-Diaminophenoxyethanol HCl (batch 0120022) as 5 mg/ml, 50 mg/ml and 100 mg/ml in DMSO solution was shown to be stable up to 4 hours at room temperature, protected from light and under inert gas atmosphere.

General comments on physico-chemical characterisation

- Stability of 2,4-diaminophenoxyethanol in marketed products is not reported
- no data is reported on physico-chemical characterisation/properties of 2,4-diamino-phenoxyethanol sulphate

3.2. Function and uses

2,4-Diaminophenoxyethanol, an ingredient of oxidative hair colouring products is used at a maximum final (on-head) concentration of 2.0%, after mixing the hair dye formulation with a hydrogen peroxide preparation typically in 1:1 proportions.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 401
Species/strain:	Rat, Sprague Dawley ICO:OFA-SD (IOPS Caw)
Group size:	One group of 5 males and 5 females
Test substance:	Imexine OAJ, test substance 2952
Batch:	0101297
Purity:	99.6%
Dose:	1000 mg/kg
Observation:	14 days
GLP:	in compliance

The test substance was administered by oral (gavage) to one group of ten fasted Sprague Dawley rats (5 males and 5 females) at a dose of 1000 mg/kg bw.
Clinical signs and mortality were checked daily for a period of 14 days following the single administration of the test item. The animals were checked for body weight gain on day 1, 8 and 15 and were subjected to necropsy.

Results
Hypoactivity or sedation and piloerection were noted in all animals on day 1. Lateral recumbency and tonic-clonic convulsions were also observed in two animals on day 1, one of these animals was found dead a few hours later. One male and two females were found dead on day 2. Hypoactivity or sedation and piloerection persisted in the surviving animals up to day 3 or 4; dyspnoea and unsteady gait were recorded in the surviving females up to day 3.
Body weight gain in surviving animals was not affected by treatment with the test substance. No apparent abnormalities were observed at necropsy in all animals.

Conclusion
Under the experimental conditions, the LD_{50} of the test item was close to 1000 mg/kg bw, since a single dose of 1000 mg/kg induces death in 1/5 male and 3/5 female rats.

Ref.: 1

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: OECD 404
Species: New Zealand white rabbits
Group: 3 females
Substance: IMEXINE OAJ
Batch: Op.118
Purity: 99.4%
Dose: 0.5 g over 6 cm^2 for 4 hours
GLP: in compliance

The day before application, the back area of test animals was clipped free of hair. A 0.5 g sample of neat IMEXINE OAJ was applied to 6 cm^2 of the right flank which was previously treated with 0.5 ml paraffin oil. It was held in contact with the skin for 4 hours by means of a non-occlusive dressing. The untreated left flank served as control. The dressing was then removed, and cutaneous reactions were assessed 1, 24, 48 and 72 hours after dressing removal.

Results
There were no cutaneous reactions apart from a slight erythema observed in 1 animal at the 48-hour observation only.

Conclusion
When tested undiluted, IMEXINE OAJ was non-irritant to rabbit skin.

Ref.: 2

3.3.2.2. Mucous membrane irritation

Acute Eye Irritation Study in Rabbits (neat test item)

Guideline: OECD 405
Species: New Zealand white rabbits
Group: 3 females
Substance: IMEXINE OAJ
Batch: Op.118
Purity: 99.4%
Dose: 100 mg
GLP: in compliance

On day 1, a 100 mg sample of neat IMEXINE OAJ was instilled in the conjunctival sac of the left eye of the animals. The eyes were not rinsed following instillation of the test item. The non-treated right eye served as control. The ocular reactions were assessed 1, 24, 48 and 72 hours after instillation, as well as on day 8 and day 15.

Results
Marked ocular reactions were observed. They included moderate to marked chemosis and slight to moderate redness of conjunctivae, slight to moderate corneal opacification and slight iridal lesions. At the end of the study (day 15), similar though less marked ocular reactions were observed.

Conclusion
IMEXINE OAJ was considered to be an irritant to rabbit eyes when tested undiluted.

Ref.: 3

Acute Eye Irritation Study in Rabbits (diluted test item)

Guideline: OECD 405
Species: New Zealand white rabbits
Group: 3 males
Substance: 2,4-diaminophenoxyethanol dihydrochloride
Batch: 0120022
Purity: 98.7%
Dose: 0.1 ml of 4% aqueous solution of 2,4-diaminophenoxyethanol dihydrochloride
GLP: in compliance

On day 1, a 0.1 ml aliquot of a 4% solution of 2,4-diaminophenoxyethanol dihydrochloride in purified water was instilled into the left conjunctival sac of test animals. The eyes were not rinsed following instillation of the test item. The non-treated right eye served as control. The ocular reactions were assessed 1, 24, 48 and 72 hours after instillation.

Results
Ocular reactions were limited to conjunctival reactions. They consisted of slight chemosis and slight redness observed in 2 animals which persisted to day 2 in 1 animal. There were no corneal or iridal reactions.

Conclusion
2,4-diaminophenoxyethanol dihydrochloride was slightly irritant to rabbit eyes when tested at 4% in water.

Ref.: 4

3.3.3. Skin sensitisation

Buehler Test

Guideline:	OECD 406
Species:	Dunkin-Hartley guinea pigs
Group:	15 animals of each sex
Substance:	IMEXINE OAJ
Batch:	0101297
Purity:	99.6%
Dose:	500 mg neat IMEXINE OAJ or 0.5 ml water (control)
GLP:	in compliance

The sites to be treated were clipped and/or shaved before each induction or challenge application.

The concentration of IMEXINE OAJ used for challenge and induction applications was selected on the basis of the results a preliminary irritation test where undiluted IMEXINE OAJ was non-irritant under the conditions of the study. The induction procedure consisted of 3 weekly topical applications of neat IMEXINE OAJ. On day 1, a moistened gauze pad (about 4 cm^2) dampened with 0.5 ml water or loaded with 500 mg neat IMEXINE OAJ was applied to the anterior left flank of control (5/sex) or treated (10/sex) animals, respectively. It was held in place for 6 hours by an occlusive dressing. This treatment was repeated on day 8 and day 15 of the study.
On day 29, following a 2-week rest period, all control and treated animals received under similar conditions a topical challenge application of 0.5 ml water and 500 mg neat IMEXINE OAJ on posterior left and right flank, respectively. Cutaneous reactions were assessed 24 hours after each dressing removal during the induction period, and 24, 48 and 72 hours after removing the dressing of challenge application.

Results
Cutaneous reactions were limited to purple colouration of the skin and very slight erythema in 2/20 treated animals at the 48-hour reading only for the latter finding.

Conclusion
Under the conditions of the study, the topical application of undiluted IMEXINE OAJ did not produce sensitisation reactions.

Ref.: 5

Local Lymph Node Assay

Guideline:	OECD 429
Species:	CBA/J mice
Group:	28 females; 7 groups of 4 mice
Substance:	2,4-diaminophenoxyethanol dihydrochoride
Batch:	0120022
Purity:	98.7%
Dose:	0.5, 1, 2.5, 5 and 10 % w/v in dimethylsulphoxide (DMSO)
GLP:	in compliance

Animals were separated in 7 groups (4 mice/group) consisting of:

- 5 treated groups receiving 2,4-diaminophenoxyethanol dihydrochoride at the concentrations of 0.5, 1, 2.5, 5 and 10% (w/v) in dimethylsulfoxide (DMSO). This vehicle was selected in a previous solubility study showing that 2,4-diaminophenoxyethanol dihydrochoride was non-soluble in other recommended vehicles, and that 10% (w/v) 2,4-diaminophenoxyethanol dihydrochoride in DMSO was the maximal practicable concentration. This concentration was non-irritant in a preliminary test.
- A negative control group receiving the vehicle (DMSO) alone
- A positive control group receiving alpha-hexylcinnamaldehyde at 25% (v/v) in DMSO.

The test item 2,4-diaminophenoxyethanol dihydrochoride, DMSO or alpha-hexylcinnamaldehyde was applied over the ears (25 µL per ear) of respective animals for three consecutive days designated as days 1, 2 and 3. After 2 days of resting (day 6), mice received a single intravenous injection of tritiated methyl thymidine (^{3}H-TdR). Lymph nodes draining the application sites (auricular nodes) were sampled, pooled per group, and the proliferation of lymphocytes was evaluated by measuring the incorporation of ^{3}H-TdR. The values obtained were used to calculate stimulation indices (SI), and the EC_3 was calculated (theoretical concentration resulting in a SI of 3). The irritant potential of the test item was assessed by measuring ear thickness on days 1, 2, 3 and 6.

Results
A SI value of 8.5 was obtained in the alpha-hexylcinnamaldehyde positive control group. This value was indicative of lymphoproliferation and showed the adequate sensitivity of the test system and procedure used.
In 2,4-diaminophenoxyethanol dihydrochoride groups, a dose-related increase in stimulation index was observed, and the threshold positive value of 3 was exceeded at concentrations of 5 and 10%. In the absence of local irritation attributed to 2,4-diaminophenoxyethanol dihydrochoride, these lymphoproliferative responses were attributed to delayed contact hypersensitivity, and the EC_3 value was calculated to be 3.2%.

Conclusion
Under the conditions of this study, 2,4-diaminophenoxyethanol dihydrochoride induced delayed contact hypersensitivity in the murine Local Lymph Node Assay. Based on the EC_3 value obtained (3.2%), it was considered to be a moderate skin sensitiser.

Ref.: 6

3.3.4. Dermal / percutaneous absorption

***In Vitro* Percutaneous Absorption Study using Human Dermatomed Skin**

Guideline:	OECD draft 428
Species:	Human
Group:	7 female donors, breast and abdominal skin
Substance:	2,4-diaminophenoxyethanol dihydrochoride
Batch:	0120022
	CFQ13910 Batch 1 of 2-(2,4-diamino[ring-U-^{14}C]phenoxy)ethanol HCL (98.6% radiochemical purity)
Purity:	98.7%
Dose:	2 %
GLP:	in compliance

Human breast and abdominal skin samples were obtained from seven female donors subjected to plastic surgery. The skin was transferred stored on ice and kept frozen at –20°C until required.

Skin samples were dermatomed (370-400 µm in thickness) and mounted in diffusion cells, using calcium and magnesium-free phosphate-buffered saline as the receptor fluid. The integrity of the skin was checked by determination of the permeability coefficient for tritiated water (<2.5 x 10^{-3} cm/h for all selected membranes). Twenty-four diffusion cells were used in two separate experiments, and skin was maintained at approximately 32°C.

In a first experiment (oxidative conditions), a typical oxidative hair dye formulation containing 4.0% 2,4-diaminophenoxyethanol dihydrochoride (coupler) associated with the primary intermediate p-phenylenediamine (PPD) at 1.8% was mixed with the developer (1:1, w/w) to yield a final concentration of 2.0% 2,4-diaminophenoxyethanol dihydrochoride. An aliquot of 20 mg/cm² of this mixture was applied to the skin surface and left for 30 minutes (corresponding to exactly 428 µg/cm² of 2,4-diaminophenoxyethanol dihydrochoride). After this time period, the remaining formulation on the skin surface was removed using a standardized washing procedure. Twenty-four (24) hours after application, the percutaneous absorption of [^{14}C]- 2,4-diaminophenoxyethanol dihydrochoride was estimated by measuring its concentration by liquid scintillation counting (following combustion for non-liquid samples) in the following compartments: skin washes, *stratum corneum* (isolated by tape strippings), living epidermis/dermis, unexposed skin and receptor fluid.

In a separate experiment, a similar experimental procedure was applied to evaluate the percutaneous absorption of 2,4-diaminophenoxyethanol dihydrochoride in non-oxidative conditions, using a formulation devoid of PPD containing 2,4-diaminophenoxyethanol dihydrochoride at 4.0% before mixing with water (1:1, w/w).

Results

Eight out of twelve (8/12) and 12/12 diffusion cells yielded data that could be analysed in oxidative and non-oxidative conditions, respectively. Most of the 2,4-diaminophenoxyethanol dihydrochoride applied on the skin surface was removed with the skin wash (extractable dose, about 90% and 94% of the applied dose in oxidative and non-oxidative conditions, respectively),

and the total recovery rate was about 95% and 101% in oxidative and non-oxidative conditions, respectively.

The mean amounts of 2,4-diaminophenoxyethanol dihydrochoride considered as absorbed (dermal delivery) were estimated as follows (sum of amounts measured in living epidermis/dermis and receptor fluid): 1.74 ± 1.08 µg equiv/cm^2 (0.41 ± 0.26% of the applied dose) and 6.55 ± 4.72 µg equiv/cm^2 (1.68 ± 1.23% of the applied dose) in oxidative and non-oxidative conditions, respectively.

	Oxidative conditions		**Non-oxidative conditions**	
Cutaneous Distribution	**µg equiv/cm²**	**% applied dose**	**µg equiv/cm²**	**% applied dose**
Extractable dose	379.97 ± 24.54	89.68 ± 4.06	369.63 ± 21.37	94.11 ± 4.54
Unabsorbed dose*	399.60 ± 26.01	94.31 ± 4.23	390.47 ± 13.49	99.44 ± 2.79
Receptor fluid	0.11 ± 0.12	0.03 ± 0.03	2.94 ± 3.30	0.75 ± 0.84
Dermal delivery**	1.74 ± 1.08	0.41 ± 0.26	6.55 ± 4.72	1.68 ± 1.23

* extractable dose + *stratum corneum* + unexposed skin
** Receptor fluid + living epidermis/dermis

Conclusion
The amounts of 2,4-diaminophenoxyethanol dihydrochoride considered as absorbed from a typical oxidative hair colouring mixture containing 2,4-diaminophenoxyethanol dihydrochoride at a final concentration of 2.0% were estimated to be 1.74 ± 1.08 µg/cm^2 (0.38 – 4.33 µg/cm^2).
The amounts of 2,4-diaminophenoxyethanol dihydrochoride considered as absorbed from a non-oxidative mixture containing 2,4-diaminophenoxyethanol dihydrochoride at a final concentration of 2.0% were estimated to be 6.55 ± 4.72 µg/cm^2 (1.56 – 16.61 µg/cm^2).

However, the maximum dermal absorption of 4.33 µg equiv/cm², observed under oxidative conditions in a typical hair dye formulation, will be used for the calculation of the Margin of Safety.

Ref.: 16

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline: OECD 408
Species/strain: Rat, Sprague Dawley, Crl CD (SD) IGS BR
Group size: 10 animals per sex and dose; 6 additional animals per sex for the high dose + control group (which were kept for a 4-week treatment free period) and 6 animals per sex in the satellite groups (low, mid and high dose)
Observation: 13 weeks (+4 weeks recovery period)

Test substance:	2,4-diaminophenoxyethanol
Batch:	0120022
Purity:	98.7%
Dose:	0, 4, 20, 100 mg/kg bw/day
GLP:	in compliance

A total of 140 Sprague-Dawley rats (70 males and 70 females) were allocated to three treated groups and one control group. Each group was composed of 10 animals/sex. Recovery animals (six animals/sex) were added to the control and high-dose groups for a 4-week treatment-free period. Satellite animals (six animals/sex) were allocated to each treated group for toxicokinetic investigations. The test substance was administered daily for 13 weeks, by gavage, as a solution in the vehicle (purified water), at the dose-level of 4, 20 or 100 mg/kg/day. Control animals received the vehicle only under the same experimental conditions.
The animals were checked daily for mortality and clinical signs. Detailed clinical observations were carried out weekly and a functional observation battery (including motor activity) was conducted at the end of the treatment period. Body weight and food consumption were recorded once a week during the study.
Ophthalmological examinations were performed before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations as well as urinalysis were performed at the end of the treatment period. Some parameters were selected to be analyzed at the end of the treatment-free periods.
Blood samples for the determination of plasma levels of the test item were taken on day 1 and in week 13 from satellite animals at designated time-points. On completion of the treatment or treatment-free period, the animals were sacrificed and submitted to a full macroscopic examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from animals in the control and high-dose groups and on macroscopic lesions from all animals killed on completion of the treatment period. In addition, the thyroids and spleen from all animals of the low and mid-dose groups and from those killed on completion of the treatment-free period were microscopically examined.

Results
No unscheduled deaths occurred during the study. There were no perturbations of the autonomic or physiological functions in any treated group. Ptyalism was noted among almost all the animals given 100 mg/kg/day.
A slightly lower mean body weight gain was noted in males given 100 mg/kg/day during the whole dosing period and was most of the time statistically significant. Mean male body weight returned to control values during the treatment-free period.
No treatment-related effect was noted on females body weight gain.
No treatment-related effects were noted on food consumption for either males or females, and there were no ophthalmological treatment-related findings at the end of the treatment period.
No treatment-related changes were noted for any haematological or blood biochemical parameters. At the end of the treatment period, the following treatment-related differences from controls were observed in urinary parameters at 100 mg/kg/day:

- presence of low to high bilirubin levels in all males and in 7/10 females,
- traces of nitrites in 8/10 males and 6/10 females,
- traces of glucose in 6/10 males and 6/10 females,
- marked coloration of urine (from yellow to yellow-brown) in both males and females.

All these changes were attributed to treatment and were no longer present on completion of the treatment-free period. However, as there were no changes in plasma levels of bilirubin and glucose, the positive reaction of urinary test strips to glucose and bilirubin at 100 mg/kg/day (and most probably also to nitrite) was likely related to an analytical interference due to the renal elimination of 2,4-diaminophenoxyethanol HCl material, as indicated by the marked coloration of urine at this dose-level.
Plasma concentrations of 2,4-diaminophenoxyethanol HCl were below the limit of quantification at 4 mg/kg/day. At 20 mg/kg/day, measurable plasma levels were found on both day 1 and in week 13 at 0.5 hours post-dosing. At 100 mg/kg/day, test item was found at significant levels on day 1 and in week 13. On both occasions, the maximum mean plasma levels were measured at 0.5 hours post-dosing (first sampling time-point). Thereafter, the plasma levels decreased steadily until the last quantifiable time-point (2 hours on day 1, 8 hours in week 13). There were no apparent sex-related differences.
Systemic exposure (as measured by AUC0-t) increased with dose-level in a proportional manner, and mean AUC0-t values of 5.22 and 7.40 µg.h/ml were achieved in week 13 for males and females given 100 mg/kg/day, respectively.
Relative kidney weights were increased in the 100 mg/kg/day group in females (significant increase) and males. Relative thyroids weight was increased at the intermediate dose level in females and the highest dose level in both males and females. Since there was no effect on the thyroids at microscopic examination, the increase in thyroids weight in the females of the intermediate dose level was not considered to be a toxicologically relevant effect.
Brownish coloration of the thyroids was observed in all males and females given 100 mg/kg/day and was associated at microscopic examination with brownish pigment deposits in the thyroids (mainly in follicular epithelial cells). There were no associated inflammatory, degenerative or proliferative changes. Spleen hemosiderosis was also observed for all animals given 100 mg/kg/day. Both these microscopic changes on thyroids and spleen were still observed at the end of the recovery period.

Conclusion
The test item 2,4-diaminophenoxyethanol HCl was administered daily by gavage to Sprague-Dawley rats at the dose-level of 4, 20 or 100 mg/kg/day for 13 weeks. At 4 and 20 mg/kg/day, the test item was well tolerated.
At 100 mg/kg/day, ptyalism was observed in both males and females and lower body weight gains were noted for males. Presence of urinary bilirubin, nitrites, glucose and coloured urine in both males and females was observed at the end of the treatment period. After a 4-week treatment-free period, all the above-mentioned changes were no longer noted. Deposition of brownish pigment in the thyroids and an augmented degree of spleen hemosiderosis were also observed for most animals given 100 mg/kg/day on completion of treatment and treatment-free periods.
Consequently, under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) is 20 mg/kg/day.

Ref.: 7

3.3.5.3. Chronic (> 12 months) toxicity

See 3.3.7 Carcinogenicity

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA102
Replicates:	Two independent tests
Test substance:	2,4-Diaminophenoxyethanol HCl
Batch:	0120022
Purity:	> 99.5%
Concentrations:	1.6 - 5000 μg/plate with and without S9 mix (1.3 - 5000 μg/plate for strain TA102 in the absence and presence of S9.
GLP:	in compliance

2,4-Diaminophenoxyethanol HCl was investigated for the induction of gene mutations in *Salmonella typhimurium*. Liver S9 fraction from rats induced with Aroclor 1254 was used as the exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline. Concentrations were defined by a preliminary toxicity range-finder study in TA100 only.

Results
No clear evidence of toxicity was observed in any of the test strains in the first experiment (1.6 - 5000 μg/plate), but the data were considered to be acceptable for mutation assessment. A statistically significant increase in revertants was observed in strains TA98 and TA102 in the presence of S9 mix. In the second experiment, different dose intervals were used for strains TA100, TA1535 and TA1537 (20.48 - 5000 μg/plate), strain TA98 (8.192 - 5000 μg/plate), and strain TA102 (1.3 - 5000 μg/plate); in the presence of metabolic activation a pre-incubation step was used. For strains TA98 and TA102 in the presence of S9, plate-incorporation treatments were additionally included to assess the reproducibility of increases in revertants observed in experiment 1. Following these treatments, evidence of toxicity was observed in strain TA102 only in the presence of S9 for pre-incubation and plate-incorporation treatments. A statistically significant and dose-related increase in revertants was observed for TA98 in the presence of S9 mix also in the second experiment. As an increase was not observed for TA102 in the second experiment, the initial positive effect was considered to be due to a chance event.

Conclusion
2,4-Diaminophenoxyethanol HCl induced mutations in *Salmonella typhimurium* strain TA98 in the presence of S9 when tested under the conditions of this study.

Ref.: 8

***In vitro* mammalian cell gene mutation test (HPRT)**

Guideline:	OECD 476
Cells:	L5178Y mouse lymphoma cells (HPRT)
Replicates:	two independent tests with and without S9 mix
Test substance:	2,4-Diaminophenoxyethanol HCl
Batch:	0120022
Purity:	> 99.5%
Concentrations:	400 – 2410 µg/ml without metabolic activation, 3 h 200 – 1800 µg/ml with metabolic activation, 3 h
GLP:	in compliance

2,4-Diaminophenoxyethanol HCl was investigated for the induction of gene mutations at the HPRT-locus in L5178Y mouse lymphoma cells after exposure for 3 hours with and without metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Test concentrations were based on the level of toxicity. Negative and positive controls were in accordance with the OECD guideline.

Results
In the cytotoxicity range-finding experiment, extreme toxicity (<3% relative survival) was observed at the highest dose tested (2410 µg/mL). In the first experiment, the highest doses selected, 2200 µg/ml in the absence of S9 mix and 1800 µg/ml in the presence of S9 mix, yielded 8% and 6% relative survival, respectively. In the second experiment, the highest dose tested (2410 µg/ml) yielded 28% and 9% relative survival in the absence and presence of S9, respectively. No statistically significant increases in mutant frequency were observed following treatment with 2,4-diaminophenoxyethanol HCl in the absence of S9 mix. In the presence of S9 mix, a small but statistically significant increase in mutant frequency were observed at 1000 and 1600 µg/ml 2,4-diaminophenoxyethanol HCl in the first experiment and at 400, 1600 and 2410 µg/ml in the second experiment. However, there was no evidence of a statistically significant linear trend in experiment 1 and only a weak linear trend in experiment 2, and all mutant frequencies observed for the treated cultures fell within historical negative control range (mean ±2 standard deviations).

Conclusion
The report considered the positive signs as chance events with little or no biological relevance, concluding that, under the conditions employed in this study, 2,4-diaminophenoxyethanol HCl is not mutagenic in this test system. The result is considered equivocal.

Ref.: 9

***In vitro* chromosome aberration test**

Guideline:	OECD 473
Cells:	human lymphocytes
Replicates:	two independent tests with and without S9 mix
Test substance:	2,4-Diaminophenoxyethanol HCl
Batch:	0120022
Purity:	98.7%
Concentrations:	987.1, 1234, 1542 µg/ml with and without metabolic activation, treatment for 3 h; 1408, 1760, 2200 µg/ml with metabolic activation, treatment for 3 h; 49.54, 77.41, 96.76, and 120.9 µg/ml, 20 h continuously, without metabolic activation

GLP: in compliance

2,4-Diaminophenoxyethanol HCl was investigated for the induction of chromosomal aberrations in cultured human lymphocytes with and without metabolic activation by liver S9 fraction from Aroclor1254-induced rats. Concentrations were chosen for scoring based upon reduction in mitotic index. Negative and positive controls were in accordance with the OECD guidelines.

Results
The highest concentration chosen for analysis in the first experiment (1542 μg/ml) induced approximately 54% and 55% mitotic inhibition (reduction in mitotic index) in the absence and presence of S9 mix, respectively. In the second experiment, the highest concentrations chosen for analysis, 120.9 μg/ml in the absence of S9 and 2200 μg/ml in the presence of S9 mix, induced approximately 66% and 39% mitotic inhibition, respectively. The intermediate concentration applied in the presence of S9 (1760 μg/ml) showed 44% mitotic inhibition. In the second experiment, the 3-h treatment with 2,4-diaminophenoxyethanol HCl in the presence of S9 induced an increase in structural chromosome aberrations ($p \leq 0.001$, compared with concurrent solvent control cultures). In experiment 1, where the concentration range was lower, no statistically significant increases were observed following the 3-h treatment with S9 mix. Also the 20-h continuous treatment with 2,4-diaminophenoxyethanol HCl in the absence of S9 mix resulted in a significant elevation of chromosome aberrations compared with the concurrent solvent control cultures.

Conclusion
2,4-Diaminophenoxyethanol HCl induced chromosome aberrations in cultured human peripheral blood lymphocytes, when tested in the absence or presence of a rat liver metabolic activation system.

Ref.: 10

***In vitro* micronucleus test**

Guideline:	OECD draft guideline 487
Cells:	Human lymphocytes
Replicates:	2 independent tests with and without S9 mix, 24 and 48 hours after PHA stimulation
Test substance:	2,4-Diaminophenoxyethanol HCl
Batch:	0120022
Purity:	> 99.5%
Concentrations:	106-165.6 μg/ml without metabolic activation for 20 h, 24 after PHA stimulation, 28-h recovery; 160.3 - 361.3 μg/ml without metabolic activation; treatment for 20 h, 48 h after PHA stimulation, 28-h recovery; 84.79 - 1542 μg/ml with metabolic activation for 3 h, 24 or 48 h after mitogen stimulation, 45-h recovery; 1542 - 2410 μg/ml with metabolic activation for 3 h, 24 or 48 h after mitogen stimulation, 45-h recovery.
GLP:	in compliance

2,4-Diaminophenoxyethanol HCl was investigated for the induction of micronuclei in cultured human lymphocytes with a harvest time of 48 hours (20-h exposure without metabolic activation, 3-h exposure with metabolic activation). Liver S9 fraction from Aroclor1254-induced

rats was used as the exogenous metabolic activation system. Toxicity was determined by measuring reduction in replication index (RI). Negative and positive controls were used in accordance with the draft of the OECD guideline.

Results
The highest concentrations tested, 165.6 µg/ml in the absence of S9 and 1542 µg/ml in the presence of S9, induced approximately 52% and 64% reduction in RI, respectively. In the second experiment, the highest concentrations chosen for analysis, 361.3 µg/ml in the absence of S9 mix and 2410 µg/ml in the presence of S9 mix, induced approximately 56% and 31% reduction in RI, respectively. In treatments performed 24 h after PHA stimulation, 2,4-diaminophenoxyethanol HCl did not increase micronuclei in the absence and presence of metabolic activation. Treatment of cells with 2,4-diaminophenoxyethanol HCl 48 h after mitogen stimulation in the absence and presence of S9, resulted in a statistically significant increase in frequencies of micronucleated binucleate cells either at all concentrations analysed (20 h treatment –S9 treatment) or the highest concentration (3 h treatment +S9 mix).

Conclusion
2,4-Diaminophenoxyethanol HCl induced micronuclei in cultured human peripheral blood lymphocytes in the presence and absence of metabolic activation.

Ref.: 11

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Rat bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	Rat, Crl:CD (SD)BR
Group size:	5 males + 5 females
Test substance:	2,4-Diaminophenoxyethanol HCl
Batch:	0120022
Purity:	> 99.5%
Dose levels:	375, 750 and 1500 mg/kg bw (once by gavage)
Sacrifice time:	24 and 48 (highest dose group only) h after the treatment
GLP:	in compliance

2,4-diaminophenoxyethanol HCl was investigated for the induction of micronuclei in the bone marrow polychromatic erythrocytes of rats. Negative and positive controls were in accordance with the OECD guideline. Dose selection was based on a dose range-finding assay. In the micronucleus assay, the test article was formulated in water and administered once as follows.

Results
The highest dose chosen for the micronucleus assay was 1500 mg/kg, the estimated maximum tolerated dose. 2,4-diaminophenoxyethanol HCl induced signs of clinical toxicity at 1500 mg/kg. One animal treated at 1500 mg/kg was found dead. No statistically significant increases in micronucleus frequencies were observed in polychromatic erythrocytes (PCEs) from male and female rats at any dose of 2,4-diaminophenoxyethanol HCl examined. One male (48-h time-point) and one female (24-h time-point) at the 1500 mg/kg dose level had elevated micronucleus responses. However, the overall micronucleus responses were not statistically significant when compared with the concurrent vehicle control, and these isolated increases were considered to

bear no biological relevance. No statistically significant decreases in the PCE:NCE ratios (an indicator of cytotoxicity) were observed with 2,4-diaminophenoxyethanol at doses up to 1500 mg/kg in either male or female rats. Although there were no indications of bone marrow toxicity, the oral bioavailability of 2,4-diaminophenoxyethanol HCl was evidenced by the clinical signs observed at 750 and 1500 mg/kg and the death observed at 1500 mg/kg. In a contemporary 3-month toxicity study, systemic exposure to unchanged 2,4-diaminophenoxyethanol HCl was achieved in rats given a single gavage at 20 and 100 mg/kg.

Conclusion
2,4-Diaminophenoxyethanol HCl was considered negative in the rat bone marrow micronucleus test under the conditions of this assay.

Ref.: 12

Rat liver *in vivo/in vitro* UDS assay

Guideline:	OECD guideline 494
Species/strain:	Rat, Crl:CD (SD)BR
Group size:	4 or 6 males
Test substance:	2,4-Diaminophenoxyethanol HCl
Batch:	0120022
Purity:	> 99.5 %
Dose levels:	375, 750, and 1500 mg/kg bw, by gavage
Sacrifice times:	2-4 h or 14-16 h
GLP:	in compliance

2,4-Diaminophenoxyethanol HCl was investigated for the induction of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* following *in vivo* dosing. The dose levels were selected on the basis of a preliminary toxicity study. Negative and positive controls were in accordance with the OECD guideline. The animals were sacrificed after 14-16 hours and 2-4 hours.

Results
Among the animals treated with 375 mg/kg of 2,4-diaminophenoxyethanol HCl at the 14-16 hour time point, high percentages of nuclei with ≤5 net nuclear grains occurred in two out of three animals, and one of the responding animals had a response in only one out of three slides. In the other groups, UDS response was similar to that obtained for the vehicle control animals for both harvest times. Based on the inconsistent results across animals and slides, and the observation that the response was not dose-related, the slight changes at 375 mg/kg were considered spurious and unrelated to the treatment.

Conclusion
2,4-Diaminophenoxyethanol HCl was evaluated as negative in the *in vivo/in vitro* assay for UDS in the livers of male rats under the conditions of this study.

Ref.: 13

3.3.7. Carcinogenicity

Guideline: /

Species/strain:	RatsF344/DuCrj and Mice, CRJ:BDF1
Group size:	Rat study: groups of 50 mice/sex/dose level
Mice study:	groups of 50 mice/sex/dose level
Observation:	104 weeks
Test substance:	2-(2', 4'-Diaminophenoxy) ethanol dihydrochloride
Purity:	96% (stated in the text; no supportive data)
Dose:	rats: 0.1 and 0.05% 2-(2', 4'-Diaminophenoxy) ethanol dihydrochloride solution in tap water Mice: 0.07 and 0.04% 2-(2', 4'-Diaminophenoxy) ethanol dihydrochloride solution in tap water; no batch nr available
GLP statement:	not in compliance

In a preliminary study (data not provided) the MTD was determined to be 0.1% in drinking water for rats and 0.07% for mice.

Rat study

Groups of rats were allowed free access to drinking water containing 0, 0.05 or 0.1% 2-(2', 4'-Diaminophenoxy) ethanol dihydrochloride. Corresponding intake levels were 0, 20.9 and 35.5 mg/kg bw/day for male rats and 0, 27.8 and 60.9 mg/kg bw for female rats. In the high dose groups, dosing was withheld in males during weeks 12-16 and 32-36 and in females during weeks 32-36 since a marked depression of body weight gain was noted.

All animals were observed for clinical signs daily, and were weighed at 4-weeks intervals. Blood biochemical tests were performed (not clear which ones; data not reported). At necropsy, organ weight of several organs was recorded (data not reported). Microscopic examinations were performed on brain, salivary glands, lungs, heart, liver, stomach, duodenum, pancreas, spleen, kidneys, prostate, testes, seminal vesicle, urinary bladder, uterus, ovaries, pituitary, thyroids and adrenals.

Results

Body weight gain was depressed in both treatment groups, for both males and females. Overall survival rate was 71%. No results on organ weight and biochemical tests are presented in the test report (there is only a reference to a Japanese report).

In general, tumour incidences in control and treated groups were the same. In the highest dose groups, pigment deposition in the epithelial cells of thyroid follicles of both males and females was observed. The distribution of these deposits did not show a correlation with the occurrence of tumours. An increase of the C-cell adenoma was observed in the thyroid gland of male rats (1/48, 11/49 (p=0.004) and 7/11 (p=0.05)).

Conclusion

Although the study authors report that C-cell adenomas are common in old F33 rats, they do not provide historical control values. Therefore it cannot be concluded that the increase in these tumours is toxicologically significant or not. At this moment, no conclusion on the carcinogenicity can be drawn.

Mice study
Groups of mice were allowed free access to drinking water containing 0, 0.04 or 0.07% 2-(2', 4'-Diaminophenoxy) ethanol dihydrochloride. Corresponding intake levels were 0, 35.8 and 62.8 mg/kg bw/day for male rats and 0, 44.6 and 81.4 mg/kg bw for female mice.
All animals were observed for clinical signs daily, and were weighed at 4-weeks intervals. Blood biochemical tests were performed (not clear which ones; data not reported). At necropsy, organ weight of several organs was recorded (data not reported). Microscopic examinations were performed on brain, salivary glands, lungs, heart, liver, stomach, duodenum, pancreas, spleen, kidneys, prostate, testes, seminal vesicle, urinary bladder, uterus, ovaries, pituitary, thyroids and adrenals.

Results
No effect on body weight gain was noted in this study. Overall survival rate was 72%. No results on organ weight and biochemical tests are presented in the test report (there is only a reference to a Japanese report).
Tumour incidences in control and treated groups were the same. In the highest dose groups, pigment deposition in the epithelial cells of thyroid follicles of both males and females was observed. The distribution of these deposits did not show a correlation with the occurrence of tumours.

Conclusion
Under the conditions of the study, the test substance was not carcinogenic in mice.

Ref.: 14

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Prenatal development toxicity study

Guideline:	OECD 414
Species/strain:	Rat, Sprague Dawley, Crl CD (SD) IGS BR
Group size:	24 females / dose level
Observation period:	20 days
Test substance:	2,4-Diaminophenoxyethanol HCl (in purified water)
Batch:	0120022
Purity:	98.7%
Dose levels:	0, 4, 20, 125 mg/kg bw/day
GLP:	in compliance

Four groups of 24 pregnant rats received 2,4-Diaminophenoxyethanol HCl by oral gavage at doses of 0, 4, 20 or 125 mg/kg bw/day from day 6 through day 19 post-coitum. The day of positive proof for sperm in the vaginal smear or sperm plug was designated as day 0 post coitum (p.c.) or gestation day 0.

Animals were checked daily for clinical signs. Food consumption and body weight were recorded at designated intervals during pregnancy.
On day 20 post-coitum, the dams were sacrificed and subjected to a macroscopic examination. The gravid uterus was weighed and the foetuses were removed by hysterectomy. The following litter parameters were recorded: number of corpora lutea, number and distribution of implantation sites, uterine scars, early and late resorptions, dead and live foetuses. The foetuses were weighed, sexed and subjected to external, soft tissue or skeletal examinations.

Results
Maternal data
There were no premature deaths during the study. Nine females in the high dose groups were observed with excessive salivation from approximately day 15 of gestation until the end of treatment on day 19. Two other females of this group showed excessive salivation on day 18 or 19 only. Body weight gain was statistically significantly reduced throughout the dosing period resulting in an overall reduction of 24% during the dosing period (days 6 to 20 p.c.), when compared to controls ($p<0.001$). The group mean food consumption was statistically significantly reduced throughout the dosing period, when compared to controls.
No clinical signs were observed in the females of the low and intermediate dose groups.

Litter data
There was a statistically significant reduction in the mean foetal weight (-7%, $p < 0.001$) at 125 mg/kg bw, associated with a statistically significantly increased incidence of foetuses showing incomplete ossification of thoracic vertebra centrum or supernumerary short 14^{th} rib.

Conclusion
Under the experimental conditions, the No Observed Adverse Effect Levels (NOAEL) for maternal toxicity and embryo-foetal development are 20 mg/kg/day.

Ref.: 15

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(2,4-Diaminophenoxyethanol HCl)
(Oxidative/permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**4.33 µg/cm^2**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**3.031 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.051 mg/kg**
No observed adverse effect level (mg/kg) (rat, 13 week, oral)	**NOAEL**	**=**	**20 mg/kg**

Margin of Safety	**NOAEL / SED**	**=**	**392**

3.3.14. Discussion

Physico-chemical specification
The stability of 2,4-diaminophenoxyethanol HCl in marketed products is not reported. No data are reported on the physico-chemical characterisation/properties of 2,4-diamino-phenoxyethanol sulphate.

General toxicity
The LD_{50} of the test item was close to 1000 mg/kg bw. The NOAEL was set at 20 mg/kg bw/day in a rat 13-week toxicity study and in a rat prenatal development toxicity study.

Irritation / sensitisation
The test substance was not irritant to rabbit skin. It was slightly irritant to rabbit eyes when tested undiluted or at 4% in water.
The topical application of undiluted 2,4-diaminophenoxyethanol dihydrochoride did not produce sensitisation reactions in a Buehler test. However, it was considered to be a moderate skin sensitiser in an LLNA study.

Dermal absorption
The maximum dermal absorption, observed under oxidative conditions in a typical hair dye formulation, was 4.33 µg equiv/cm².

Mutagenicity
2,4-diaminophenoxyethanol HCl was mutagenic *in vitro*. It induced gene mutations in *Salmonella typhimurium* strain TA98 in the presence of metabolic activation, and chromosome aberrations and micronuclei in cultured human lymphocytes with or without metabolic activation. In mouse lymphoma L5178Y cells *in vitro*, statistically significant increases in gene mutations (HPRT) were observed in both experiments conducted, but the increase was small and dose-response was not obvious. Thus, the result is considered equivocal. The test substance did

not induce micronuclei in bone marrow erythrocytes or DNA damage (measured by unscheduled DNA synthesis) in rat liver cells *in vivo*, although both assays showed slight effects in individual animals.

In published literature, negative results have been reported for 2,4-diaminophenoxyethanol for the induction of reversion mutations in Salmonella typhimurium strains TA1535, TA100, TA1537, TA1538, and TA98 with and without Aroclor-1254-induced rat-liver microsomal activation system and for the induction of reverse mutations in strain XV185-14C and gene conversions in strain D4 of the yeast *Saccharomyces cerevisiae* (Shahin et al. 1980, 1982; Kalopissis 1981). The urine of rats treated topically, orally or intraperitoneally with 2,4-diaminophenoxyethanol showed no mutagenic activity (Shahin et al. 1980).

Carcinogenicity
The test substance was not carcinogenic in a mouse 104-week drinking water study. No conclusion can be drawn from the rat study.

4. CONCLUSION

The SCCP is of the opinion that the use of 2,4-diaminophenoxyethanol HCL itself as an oxidative hair dye at a maximum concentration of 2.0 % in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitising potential.

No data were submitted on 2,4-diaminophenoxyethanol sulphate.

Studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

6. REFERENCES

References in italics are not submitted as full reports in the present dossier [18-26]. They consist of reports for studies which were considered to be inadequate for submission and reports for preliminary toxicity studies; they can be provided upon request. Appropriate data bases were searched for relevant safety data on 2,4-DAPE. No reports were identified in the literature that provided new information which is reasonably expected to substantially alter the human risk assessment performed in the present submission. In addition, the majority of published studies were performed with test articles of unknown purity and/or impurity profile, which does not permit to put the results into proper perspective. Therefore, the studies were not included in the present submission. However, results of the literature search can be provided upon request.

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