Annex VI Dossier

Proposal for Harmonised Classification and Labelling

Substance name: Leucomalachite Green
EC Number: 204-961-9
CAS Number: 129-73-7

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Background to Proposal:

Classification and labelling for leucomalachite green was discussed and agreed by the Technical Committee on Classification and Labelling (Directive 67/548/EEC) ('TC C&L') between 2005 and 2007. The scientific assessment could therefore be regarded as having being finalised at an EU expert level. Summary records of the meetings at which the human health and environmental classifications of leucomalachite green were discussed are attached to this Annex VI dossier.

However, these agreed classifications were not formally adopted by the Commission for inclusion into Annex I of Directive 67/548/EEC before the introduction of the CLP Regulation. A proposal is therefore required in line with Articles 36 to 38 of the CLP regulation for the classification of this substance to be harmonised.

This proposal aims to formalise the classification and labelling of this substance in line with ECHA Document RAC/07/2009/40 recommending an accelerated and smooth procedure for the adoption of these classifications. The information presented below is exactly the same as that on which the classification and labelling was agreed by the TC C&L.

Proposal for Harmonised Classification and Labelling

Substance Name:	Leucomalachite Green
EC Number:	204-961-9
CAS Number:	129-73-7
Registration number:	There is no registration number for this substance at this time.
Purity:	Leucomalachite Green is marketed in a pure form (≥ 95%) with no information on impurities. The purity of leucomalachite green is therefore considered to be 95-100%.
Impurities:	No significant impurities are known.

Proposed classification based on Directive 67/548/EEC criteria:

Muta. Cat. 3; R68
Carc. Cat. 3; R40

Proposed classification based on CLP criteria:

Muta. 2; H341
Carc. 2; H351

Proposed labelling:

Directive 67/548/EEC: Class of danger: Harmful (Xn),
R: 40-68
S: (2-)36/37-46-60-61

CLP Regulation: Pictogram: , GHS08, Signal word: Warning
Hazard statement codes:, H341, H351, Precautionary statements

Proposed specific concentration limits:

Proposed notes:

None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE

Name: Leucomalachite Green

EC Number: 204-961-9

CAS Number: 129-73-7

IUPAC Name: N,N,N',N'-Tetramethyl-4-4'-benzylidenedianiline

1.2 COMPOSITION OF THE SUBSTANCE

Chemical Name: Leucomalachite Green

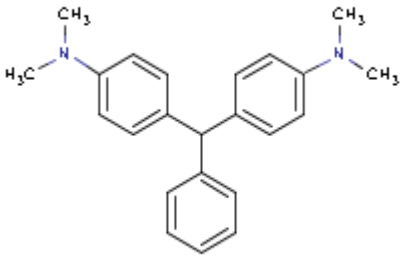
EC Number: 204-961-9

CAS Number: 129-73-7

IUPAC Name: N,N,N',N'-Tetramethyl-4-4'-benzylidenedianiline

Molecular formula: $C_{23}H_{26}N_2$

Structural formula:



Molecular Weight: 330

Typical concentration: 98 % w/w (no significant impurities are known)

Concentration range: 95 to 100 % w/w

Synonyms: N,N,N',N'-Tetramethyl-4-4'-benzylidenedianiline, leucomalachite green

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 1.1 Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value
VII, 7.1	Physical state at 20°C and 101.3 KPa	4.1	Solid (faint green colour)
VII, 7.2	Melting/freezing point	4.2	100°C

No further information is available.

2 MANUFACTURE AND USES

2.1 MANUFACTURE

2.2 IDENTIFIED USE

Leucomalachite green is used as a histopathology stain.

2.3 USES ADVISED AGAINST

3 CLASSIFICATION AND LABELLING

The substance is not currently classified in Annex VI of the CLP Regulation.

4 ENVIRONMENTAL FATE PROPERTIES

The environmental endpoints discussed and agreed by the TC C&L are contained within Annex III. There is no additional information readily available.

4.1 DEGREDATION

4.1.1 STABILITY

4.1.2 BIODEGREDATION

4.1.2.1 BIODEGREDATION ESTIMATION

4.1.2.2 SCREENING TESTS

4.1.2.3 SIMULATION TESTS

4.1.3 SUMMARY AND DISCUSSSION OF PERSISTENECE

4.2 ENVIRONMENTAL DISTRIBUTION

4.2.1 ADSORPTION/DESOPTION

4.2.2 VOLATILISATION

4.2.3 DISTRIBUTION MODELLING

4.3 BIOACCUMULATION

4.3.1 AQUATIC BIOACCUMULATION

4.3.1.1 BIOACCUMULATION ESTIMATION

4.3.1.2 MEASURED BIOACCUMULATION DATA

4.3.2 TERRESTRIAL BIOACCUMULATION

4.3.3 SUMMARY AND DISCUSSION OF BIOACCUMULATION

4.4 SECONDARY POISONING

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 TOXICOKINETICS

There is no comprehensive toxicokinetic study available on leucomalachite green. However, the occurrence of systemic toxicity and DNA adducts in the liver of rats after oral dosing indicates that leucomalachite green is absorbed via the gastro-intestinal tract to some extent.

5.2 ACUTE TOXICITY

5.2.1 ORAL

5.2.2 DERMAL

5.2.3 . INHALATION

5.2.4 SUMMARY AND DISCUSSION OF SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE

This endpoint is not covered in this proposal. Further information can however be found in the attached Annexes.

5.3 IRRITATION

5.3.1 SKIN

5.3.2 EYE

5.3.3 RESPIRATORY TRACT

5.3.4 SUMMARY AND DISCUSSION OF IRRITATION

This endpoint is not covered in this proposal. Further information can however be found in the attached Annexes.

5.4 CORROSIVITY

This endpoint is not covered in this proposal. Further information can however be found in the attached Annexes.

5.5 SENSITISATION

5.5.1 SKIN

5.5.2 RESPIRATORY SYSTEM

5.5.3 SUMMARY AND DISCUSSION OF SENSITISAITON

This endpoint is not covered in this proposal. Further information can however be found in the attached Annexes.

5.6 REPEATED DOSE TOXICITY

Two short-term repeated dose studies on leucomalachite green are available. These are summarised below and are the same data as those presented to the TC C&L. These data are provided as supporting information only and are not intended to be considered for harmonised classification.

5.6.1 ORAL

Species/strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat (Fischer 344) 8 male/dose group	0, 290, 580, and 1160 ppm in diet Leucomalachite green ≥ 98% purity	28 days	There were no significant clinical signs of toxicity (<10% bodyweight loss at the highest dose). The liver appeared to be the target organ with significantly increased relative liver weight at all 3 dose levels, an increase in the levels of γ-glutamyl transferase in the top dose group (2.2-fold greater than control, P<0.05), and slight increases in phosphorous levels in the top dose group (10% increase P<0.05). A significant dose-related trend in hepatocyte vacuolisation was observed (2/8, 5/8, 7/8 for 290, 580 and 1160 ppm dose groups respectively). Slight, but significant haematological changes were noted in the top dose group. Apoptotic follicular epithelial cells in the thyroid gland were observed at the top two doses (2/8 in 580 ppm and 2/8 in 1160 ppm). Sloughed follicular cells with condensed nuclei located within the follicles were observed. There was no inflammatory reaction, and there was evidence of follicular epithelium regeneration (Culp *et al*., 1999).
Rat (Fischer 344) 8 male/dose group	0 and 1160ppm in diet	4 or 21 days	Additional rats (8/dose /time point) were fed 0 or 1160 ppm leucomalachite green for 4 or 21 days, then T3, T4 and TSH levels were measured. A significant decrease in T4 levels and a significant increase in TSH levels was found at 4 and 21 days (T4 4 days: 5.0 and 3.4 ug/dl for control and 1160 ppm respectively, 21 days: 3.0 and 2.3 ug/dl for control and 1160 ppm respectively. TSH 4 days: 1.9 and 3.0 ng/ml for control and 1160 ppm, 21 days: 3.7 and 6.3 ng/ml for control and 1160 ppm (Culp *et al*., 1999).
Mouse ($B6C3F_1$) 8 female/dose group	0, 290, 580, and 1160 ppm in diet ≥ 98% purity	28 days	There were no significant clinical signs of toxicity (<10% bodyweight loss at the highest dose). All mice in the top dose group had scattered dead or degenerate cells in the transitional epithelium of the urinary bladder (Culp *et al.*, 1999).

5.6.2 INHALATION

There is no relevant information available on leucomalachite green or malachite green.

5.6.3 DERMAL

There is no relevant information available on leucomalachite green or malachite green.

5.6.4 SUMMARY AND DISCUSSION OF REPEATED DOSE TOXICITY

These data are provided as supporting information only.

5.7 MUTAGENICITY

Several *in vitro* and *in vivo* studies have been conducted with leucomalachite green. These are summarised below and are the same data as were presented to the TC C&L.

5.7.1 *IN VITRO* DATA

Test	Cell type	Conc. range	Metabolic activation	Observations and remarks
Ames	*Salmonella typhimurium* TA97a, TA98, TA100 and TA102	10-2000 µg/plate	+/- S9	**Negative** in all strains in a well-conducted Ames test (Fessard *et al*., 1999).
Mammalian cell gene mutation (*Hgprt*)	CHO	5-100 µg/ml	+/- S9	**Negative.** In the absence of metabolic activation mutant frequencies were repeatedly above control values at 75 ug/ml. In the presence of metabolic activation the mutant frequency at 5 ug/ml was significantly increased in one experiment. Results at other concentrations were negative. Overall results indicate a negative result (Fessard *et al*., 1999).
Comet	CHO	5-500 µg/ml	+/- S9	**Negative.** Leucomalachite green had no significant effect on cell viability and DNA in the absence (5-500 ug/ml) and presence (25-300 ug/ml) of 10-20% exogenous activation (Fessard *et al*., 1999).

5.7.2 *IN VIVO* DATA

Test	Species	Tissue	Sampling time	Observations and remarks
Gene mutation assays in				**Positive.** Mice were fed 0 or 408 ppm leucomalachite green for 16 weeks, then 10 ug DNA from each

transgenic animals **(a)** *lacII* mutation assay **(b)** Lymphocyte mutation assay *(Hprt)*	Mouse (Big Blue B6C3F1) 6 female/ group 6 animals sacrificed/ group after 28 days	Liver Spleen	16 weeks 4 and 16 weeks	animal was extracted and analysed. The degree of mutant independence for control and treated mice was similar. When *lacII* mutant frequencies were corrected for independence leucomalachite green significantly increased the incidences of liver *lacII* mutations, specifically G→T and A→T transversions. Female mice were dosed with 0, 204 ppm or 408 ppm leucomalachite green. At 4 weeks there was a significant difference among groups due to a relatively low mutant frequency in mice treated with 204 ppm leucomalachite green. No significant difference was observed between mutant frequencies in any treated group or control when analysed via Dunnets test. At 16 weeks *Hprt* lymphocyte mutant frequencies were not significantly different from controls (Mittelstaedt *et al* 2004).
Gene mutation assays in transgenic animals (a) lac I mutation assay (b) Lymphocyte mutation Assay (Hprt)	Rat (Big Blue) 6 female/ group	Liver Spleen	4, 16, 32 weeks	**Equivocal**. Doses of 0, 9, 27, 91, 272 or 543 ppm leucomalachite green was fed to female Big Blue rats for 4, 16 or 32 weeks. Lower dose groups were not analysed because there was no increase in mutant frequencies in the 91 ppm or 272 ppm groups. An approximately 3-fold increase in the lac I mutant frequency was found in the livers of rats fed 543 ppm leucomalachite green for 16 weeks. No other significant differences were noted at any other dose or time point, indicating uncertainty over the significance of this increase (Culp et al., 2002). 80 mutants from the 16 week 543 ppm group had the 1080 bp lac I gene sequenced. The liver lac I mutation frequency, when corrected for clonality, was $36x10^{-6}$ and was not significantly different from the control frequency. The predominant mutation was G:C→A:T transitions (Majanatha et al., 2004). Female rats were fed 0, 9, 27, 91, 272 or 543 ppm leucomalachite green for up to 32 weeks. None of the doses or time points showed a significant increase in Hprt mutants over the appropriate control (control group lymphocyte mutant frequencies ranged from $3x10^{-6}$ to $12x10^{-6}$, leucomalachite green-fed groups ranged from $2x10^{-6}$ to $11x10^{-6}$) (Majanatha et al., 2004).
DNA adducts	Rat (F344) 8 males/group Mouse (B6C3Fl)	Liver	28 days	**Positive**. Male rats and female mice received leucomalachite green (0, 96, or 580 ppm) in the diet for 28 days. At the end of the treatment period, DNA was isolated from the livers, and

	8 females/ group ≥ 98% purity			adduct levels measured using 32P-postlabelling with n-butanol enrichment. A single type of adduct (or co-eluting adducts) was observed in both species (although only low levels observed in mice), with adduct levels increasing significantly as a function of the dose. (Culp et al., 1999).
DNA adducts	Rat (Big blue) 4 females/group 98% purity	Liver	28 days	**Positive**. Female rats received leucomalachite green (0, 9, 27, 91, 272 or, 543 ppm) in the diet for 28 days. At the end of the treatment period, DNA was isolated from the livers, and adduct levels measured using 32P-post-labelling with n-butanol enrichment. An increase in liver DNA adduct (or co-eluting adduct) levels was observed from 92 ppm upwards. No discernible adduct was apparent in the 0, 9, or 27 ppm dose groups (Culp et al., 2002).
Micronucleus	Rat (Big Blue) 6 females/ group) 98% purity	Bone marrow	4, 16 and 32 weeks	**Negative**. Female rats were fed 0, 9, 27, 91, 272 or 543 ppm leucomalachite green for up to 32 weeks. No significant increase in the incidence of micronuclei was observed at any sampling time. (Majanatha et al., 2004).
Micronucleus	Mouse (B6C3F1) 12 females/group 6 animals sacrificed/ group after 28 days	Peripheral blood erythrocytes	4 and 16 weeks	**Negative**. Female mice were dosed with 0, 204 ppm or 408 ppm leucomalachite green, then 100 µl blood was sampled for mutations. No effect on reticulocyte or normochromatic erythrocyte peripheral blood micronucleus frequencies was observed. PCE/NCE (%) for 4 weeks: 0.11±0.01, 0.11±0.01 and 0.11±0.00 for control, 204 and 408ppm respectively, 16 weeks: 0.11±0.00, 0.12±0.01 and 0.11±0.00 for control, 204 and 408ppm respectively. Positive and negative controls gave expected results (Mittelstaedt et al 2004).

5.7.3 SUMMARY AND DISCUSSION OF MUTAGENICITY

The genotoxicity of leucomalachite green has been investigated in a number of studies, some of which are non-standard tests, including a study in transgenic animals.

Leucomalachite green tested negative in a number of standard *in vitro* (Ames test, COMET assay in CHO cells, and in a mammalian cell gene mutation assay (*Hgprt*) (all +/-S9)) and *in vivo* (two mouse micronucleus tests *in vivo* in bone marrow and blood erythrocytes following oral administration).

One gene mutation test in transgenic animals was positive (based upon liver *lacII* gene mutations), and a second gave equivocal results (based upon liver *lacI* gene mutations). ^{32}P-post-labelling studies in rats and mice exposed for 28 days in the diet demonstrated the formation of DNA adducts in the liver, thus indicating leucomalachite green's ability to covalently bind to DNA.

The findings from standard mutagenic tests do not indicate any mutagenic activity. However, mutations in genes in the liver of transgenic mice and DNA adducts in the liver of rats and mice indicate that leucomalachite green can reach and covalently bind to DNA, and can cause mutations in this organ.

In view of these findings it is considered prudent to presume that leucomalachite green is a potential *in vivo* somatic cell mutagen. Based on the criteria in the CLP Regulation, positive results in at least one *in vivo* assay in mammals, in the absence of germ cell mutagenicity, indicates that a classification as **Muta. Cat. 2 (H341)** is appropriate. These effects also meet the criteria for classification as **Muta Cat 3; R68** under Directive 67/548/EEC (evidence of mutagenic effects *in vivo* in the absence of germ cell mutagenicity or evidence that the substance or its metabolite reaches the germ cells).

Directive 67/548/EEC:	**Muta. Cat. 3; R68**
CLP Regulation:	**Muta. 2 (H341)**

5.8 CARCINOGENICITY

5.8.1 ORAL

The carcinogenicity of leucomalachite green has been investigated in mice and rats. These studies are summarised below and are the same data as were presented to the TC C&L.

Species/ strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
Rat (F344) 48/sex/ dose group	0, 91, 272 or 543 ppm (approx. 0, 5, 15, and 30 mg/kg bw/day males; 0, 6, 17, 35 mg/kg bw/day females)	104 weeks	The carcinogenicity of leucomalachite green was investigated in a standard dietary carcinogenicity study in male and female F344/N Nctr BR rats. Survival of 272 ppm males was greater than that of controls. Mean body weights of 543 and 272 ppm males and 272 and 91 ppm females were less than that of controls throughout the study. Relative liver weights were significantly increased in 272 and 543 ppm males and females (males: 34.30, 43.55, 51.69 mg organ weight/g body weight for control, 272 and 543 ppm respectively; female: 33.76, 37.87, 46.57 mg organ weight/g body weight for control, 272 and 543 ppm respectively). Relative thyroid gland weights of 543 ppm males (0.10 and 0.11 mg organ weight/g body weight for control and 543 ppm groups respectively) and females (0.11 and 0.14 mg organ weight/g body weight for control and 543 ppm respectively) were significantly increased. Non-neoplastic findings consisted of an increasing trend of thyroid gland cystic follicles in males and females (males: 0/47, 0/47, 0/48, 3/46 for control 91, 272, or 543 ppm respectively; female: 0/46, 1/46, 0/47, 2/48 for control, 91, 272, or 543 ppm respectively) and an increase in eosinophilic foci in the liver (males: 3/48, 14/47, 19/48,

			33/47 for control 91, 272, or 543 ppm respectively; female: 3/48, 12/48, 20/48, 16/48 for control, 91, 272, or 543 ppm respectively). Cystic degeneration was observed in male livers (4/48, 18/47, 13/48, 19/47 for control, 91, 272 and 543ppm respectively), and cytoplasmic vacuolization of the liver was observed in females (5/48, 5/48, 17/48, 22/48 for control, 91, 272, and 543ppm respectively). Hepatocellular adenomas were minimally increased in all male dose groups, and exceeded historical control ranges in males at 272ppm and female rats in the 91 ppm and 543 ppm dose groups. Incidences were: males 2/48 (4%), 2/47 (4%), 3/48 (6%), 2/47 (4%): females 1/48 (2%), 3/48 (6%), 0/48 (0%), 3/48 (6%) for control 91, 272 or 543 ppm respectively - not statistically significant. Historical control incidences for males are 0.7%, range 0-2%; females 0.2%, range 0-1%. Thyroid gland follicular cell adenomas or carcinomas (combined) and cysts were observed in males and females, and exceeded historical control ranges at the 543 and 91ppm group for males and the 272ppm group for females. Incidences were: males 0/47(0%), 2/47(4%), 1/48(2%), 3/46(7%) for control 91, 272 or 543ppm respectively - not statistically significant; females 0/46(0%), 1/46(2%), 2/47(4%), 1/48(2%), for control 91, 272 or 543ppm respectively - not statistically significant. Historical control incidences for males are 0.4%, range 0-2%; females 1.4%, range 0-3%. (NTP, 2005). An increasing trend in the combined incidence of mammary gland adenoma and carcinoma in female rats was observed. However, female body weight was reduced throughout the study and thus compounded the statistical power to detect treatment related increases, thus the NTP recommend these be discounted. Testicular interstitial (Leydig) cell adenoma occurred with a positive trend in males and was significantly increased in the top dose group (37/48 (77%), 42/47 (89%), 43/48 (90%), 45/47 (96%) for control, 91, 272 or 543 ppm respectively, historical control 85.7% range 69-90%; bilateral interstitial cell adenoma 22/48 (46%), 30/47 (64%), 38/48 (79%), 39/47 (83%) for control, 91, 272 or 543 ppm respectively). Incidences of mononuclear cell leukaemia were decreased in rats (males: 29/48 (60%), 16/47 (34%), 19/48 (40%), 7/47 (15%) for control, 91, 272 or 543ppm respectively, female: 17/48 (35%), 8/48 (17%), 5/48 (10%), 8/48 (17%) for control, 91, 272 or 543ppm respectively) and incidences of pituitary gland adenoma were significantly decreased in exposed male rats (30/45 (67%), 19/46 (41%), 21/48 (44%), 13/45 (29%) for control, 91, 272 or 543ppm respectively) (NTP, 2005).
Mouse (B6C3F 1) 48 female/ dose group	0, 91, 204, 408 ppm (approx. 0,13, 31, 63 mg/kg bw/day) NTP2005	104 weeks	The carcinogenicity of leucomalachite green was investigated in a dietary carcinogenicity study in female $B_6C_3F_1$/Nctr BR mice. Female mice were used because they were more sensitive in a range-finding study. Survival, mean body weights, and feed consumption were similar to that of controls. Relative kidney weights were significantly decreased in all dose groups. Non-neoplastic findings consisted of increased incidences of intracytoplasmic inclusions of the urinary bladder (14/46, 33/48,

			44/47, 44/44 for 0, 91, 204 and 408ppm respectively). The incidence of hepatocellular adenoma or carcinoma (combined) occurred with a positive trend and the incidence was significantly increased in 408ppm mice (3/47 (6%), 6/48 (13%), 6/47 (13%), 11/47 (23%) for control, 91, 204, or 408ppm respectively, historical control incidences: 6%, range 0-11%). The incidences of hepatocellular adenoma were increased although they were not statistically significant (3/47 (6%), 6/48 (13%), 5/47 (11%), 9/47 (19%) for control, 91, 204 or 408ppm respectively, historical control incidences: 4.6%, range 0-11%) (NTP2005).

5.8.2 INHALATION

No data on leucomalacite green or malachite green are available.

5.8.3 DERMAL

No data on leucomalacite green or malachite green are available.

5.8.4 SUMMARY AND DISCUSSION OF CARCINOGENICITY

The carcinogenicity of leucomalachite green by the oral route has been investigated in good quality studies in mice and rats.

The evidence of possible carcinogenicity was a statistically significant dose-related increase in hepatocellular adenoma or carcinoma (combined) in female mice (the only sex investigated), the incidence of which exceeded historical control ranges. In rats, there were no statistically significant increases in tumour incidence, although the incidence of hepatocellular adenoma and thyroid gland follicular cell adenoma or carcinoma was increased in both sexes and some incidences were above historical controls. Mechanistic studies have shown that leucomalachite green inhibits thyroid peroxidase suggesting that the thyroid tumours were induced by perturbation of thyroid hormone homeostasis. There was also an increase in interstitial (Leydig) cell adenoma of the testes, occurring with a positive trend, in F344 rats (statistically significant in the top dose group), but Leydig cell tumours in this strain of rat are not considered to be relevant for humans.

The evidence for carcinogenicity is not substantial, with limited evidence of tumour induction in the liver in mice (in a strain generally regarded as being particularly sensitive to the induction of such tumours) and only equivocal evidence of induction of liver tumours in female rats. It is recognised that this is only weak evidence for carcinogenicity, and the tumour profile is not typical for a genotoxic agent, but the statistically significant induction of tumours, with genotoxicity possibly involved in their induction, does raise some concern for carcinogenicity. An additional consideration is that the induction of liver tumours in mice was not associated with severe general toxicity.

The limited evidence of carcinogenicity indicates that a classification of **Carc. 2 (H351)** according to the CLP Regulation criteria is appropriate. Likewise, the available evidence indicates that a classification with **Carc.Cat.3; R40** under the Directive 67/548/EEC criteria

is justified

Directive 67/548/EEC:	**Carc. Cat. 3; R40**
CLP Regulation:	**Carc. 2 (H351)**

5.9 TOXICITY FOR REPRODUCTION

5.9.1 EFFECTS ON FERTILITY

No data are available on leucomalachite green or malachite green.

5.9.2 DEVELOPMENTAL TOXICITY

Malachite green is classified as Repr. Cat. 3; R63, based on limited evidence of developmental toxicity in rabbits (increased resorptions in the absence of maternal toxicity, with no malformations); there is no understanding of how malachite green caused these effects. The TC C&L decided that it was inappropriate to classify leucomalachite green for developmental toxicity on the basis of read-across because of the limited information available to allow a comparison of the toxicokinetics and toxicodynamics of the two substances; the limited evidence for malachite green-induced developmental toxicity; and the complex nature of the end point.

This is presented for information only.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

The following physicochemical endpoints are not considered in this proposal.

6.1 EXPLOSIVITY

6.2 FLAMMABILITY

6.3 OXIDISING POTENTIAL

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 AQUATIC COMPARTMENT (INCLUDING SEDIMENT)

7.1.1 TOXICITY TEST RESULTS

7.1.1.1 FISH

7.1.1.2 AQUATIC INVERTEBRATES

7.1.1.3 LONG-TERM TOXICITY TO AQUATIC INVERTEBRATES

7.1.1.4 ALGAE AND AQUATIC PLANTS

7.1.1.5 SEDIMENT ORGANISMS

7.1.1.6 OTHER AQUATIC ORGANISMS

7.1.2 CALCULATION OF PREDICTED NO EFFECT CONCENTRATION (PNEC)

7.2 TERRESTRIAL COMPARTMENT

7.3 ATMOSPHERIC COMPARTMENT

7.4 MICROBIOLOGICAL ACTIVITY IN SEWAGE TREATMENT SYSTEMS

7.5 CALCULATION OF PREDICTED NO EFFECT CONCENTRATION FOR SECONDARY POISONING (PNEC_ORAL)

7.6 CONCLUSION ON THE ENVIRONMENTAL CLASSIFICATION AND LABELLING

This is not considered as part of this proposal.

8 JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

A UK classification and labelling proposal for leucomalachite green was agreed by the Technical Committee on Classification and Labelling under Directive 67/548/EEC, held from September 2005 (environment) to September 2007 (human health). However, the ATP containing this agreed classification was not incorporated into Annex I of this Directive before the introduction of the CLP Regulation. A proposal is therefore required in line with Articles 36 to 38 of the CLP regulation for the classification of this substance to be harmonised.

The data shows that classifications of leucomalachite green for carcinogenicity and mutagenicity are appropriate.

The information presented in this dossier is identical to that on which the TC C&L came to an agreement on classification and labelling following the September 2007 meeting (Annex V).

9 REFERENCES

Culp, S. J., Blankenship, L. R., Kusewitt, D. F., Doerge, D. R., Mulligan, L. T. and Beland, F. A. (1999): Toxicity and metabolism of malachite green and leucomalachite green during short-term feeding to Fischer 344 rats and B6C3F_1 mice. *Chemico-Biological Interactions.,* **122**: 153-170.

Culp, S. J., Beland, F. A., Heflich, R. H., Benson, R. W., Blankenshio, L. R., Webb, P. J., Mellick, P. W., Trotter, R. W., Shelton, S. D., Greenlees, K. J., Manjanatha, M. G. (2002): Mutagenicity and carcinogenicity in relation to DNA adduct formation in rats fed leucomalachite green. *Mutat. Res,* **506-507**: 55-63..

Fessard, V., Godard, T., Huet, S., Mourot, A., and Poul, J. M. (1999): Mutagenicity of malachite green and leucomalachite green in *in vitro* tests. *Journal of Applied Toxicology.* **19**: 421-430.

Manjanatha, M. G., Shelton, S. D., Bishop, M., Shaddock, J. G., Dobrovolsky, V. N., Heflich, R. H., Webb, P. J., Blankenship, L. R., Bland, F. A., Greenlees, K. J., Culp, S. J. (2004): Analysis of mutations and bone marrow micronuclei in Big Blue rats fed leucomalachite green. *Mutat. Res.* **547(1-2)**: 5-18.

Mittelstaedt, R. A., Mei, N., Webb, P. J., Shaddocj, J. G., Dobrovolsky, V. N., McGarrity, L. J., Morris, S. M., Chen, F. A., Greenlees, K. J., Heflich, R. H. (2004): Genotoxicity of malachite green and leucomalachite green in female Big Blue B6C3F1 mice. *Mutat. Res.* **561(1-2)**: 127-38.

NTP Technical Report TR-527 Toxicology and carcinogenesis ctudies of malachite green and leucomalachite green in F344 rats and B6C3F1 mice (feed studies). NTP US Dept Health and Human Services (2005)

10 ANNEXES

10.1 ANNEX I

ECBI/35/05

FORM XI/396/93

Commission of the European Communities DG XI

CLASSIFICATION AND LABELLING OF DANGEROUS SUBSTANCES
Recommended form to be used for the proposed classification and labelling of Dangerous Substances in order to update Annex 1 of Directive 67/548/EEC

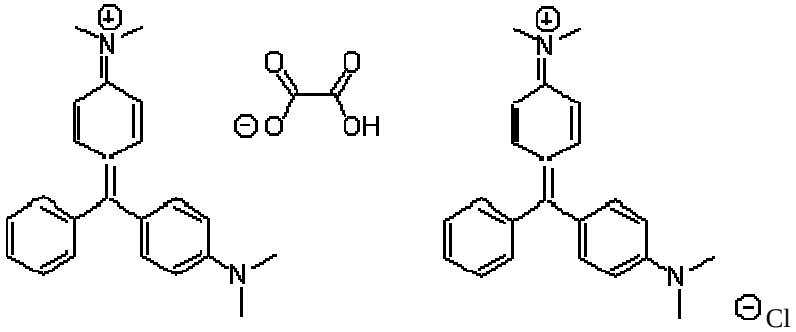
Date: June 2005	Prepared by: Health and Safety Executive, UK

The information contained in this form is not regarded as confidential

This datasheet incorporates information presented in ECBI/54/02 18th July 2002, and in addition includes further information that has become available since this time (specifically new carcinogenicity and mutagenicity data).

Data are available for both malachite green hydrochloride and malachite green oxalate have been included in this proposal as they have similar physical and chemical properties and are regarded as having equivalent toxicological hazards.

1. IDENTIFICATION OF THE SUBSTANCE

INDEX No. None	A: **EC No.** 209-322-8 **CAS No.** 569-64-2 **ID No.** 602-096-00-5 B: **EC No.** 219-441-7 **CAS No.** 2437-29-8
1.1 EINECS Name **If not in EINECS IUPAC Name**	A: [4-[alpha-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride B: bis[[4-[4-(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium] oxalate, dioxalate
1.2 Synonyms (state ISO name if available)	Malachite green hydrochloride , Malachite green chloride (A); Malachite green oxalate (B); C. I. Basic green 4; Benzaldehyde green; Acryl brilliant green; Aniline green; China green; Victoria green; Diamond green.
1.3 Molecular formula	$C_{23}H_{24}N_2HCl$ (A) $C_{48}H_{50}N_4O_4.2HC_2O_4$ (B)
1.4 Structural formula	 (B) (A)
1.5 Purity (w/w)	70-98%

1.6 Significant impurities or additives, their concentrations (w/w)	No data available
1.7 Known uses	**Industrial:** Antibacterial and antifungal agent; dye for cloth and leather; histological stain; intestinal antihelmintic; pigment in the ceramic industry; additive in the paper industry, salmon farms (now banned). **General public:** Used domestically as a treatment for diseases of tropical fish.
1.8 Proposed classification	**Muta. Cat. 3; R68 : Repr. Cat. 3; R63 : Xn; R22 : Xi; 41 : N; R50-53.**
1.9 Proposed label **Symbol**:	**R phrase(s):** 22-41-63-68-50/53 **S phrase(s):** (2-) 26-36/37/39-46-60-61 Xn; N

EXISTING LABEL	In Annex 1: Yes	Xn; R22 : Xi; R41 : Repr. Cat. 3; R63 : N; R50-53

2. PHYSICO-CHEMICAL CHARACTERISTICS

2.1 Physical form	Green crystalline powder with metallic lustre
2.2 Molecular weight	A: 365 B: 926
2.3 Melting point/range (^{o}C)	164 (B)
2.4 Boiling point/range (^{o}C)	172-175 (B)
2.5 Decomposition temperature	210 ^{o}C (B)
2.6 Vapour pressure (Pa(^{o}C))	111 at 50^{o}C
2.7 Relative density (g/ml)	1.07
2.8 Vapour density (air = 1)	16.6 (B)
2.9 Fat solubility (mg/kg, ^{o}C)	Very soluble in organic solvents
2.10 Water solubility (mg/kg, ^{o}C)	50 g/l at 80^{o}C (A)
2.11 Partition coefficient (log Pow)	No data available
2.12 Flammability **flash point (^{o}C)** **explosivity limits (%,v/v)** **auto-flammability temp. (^{o}C)**	No data available **open cup:** **closed cup:** **lower limit:** **upper limit:**
2.13 Explosivity **danger of explosion as a result of:** **explosive properties at high temperature**	No data available **shock:** **friction:** **ignition:**
2.14 Oxidising properties	No data available

2.15 Other physico-chemical properties **(eg. liberates toxic gas on heating or in contact with water or acids)**	No data available

3. OBSERVATIONS ON HUMANS

Where available, human data are considered to be of more relevance in determining the potential effects of chemical substances on the human population. (Annex V, Directive 67/548/EEC).

3.1 Occupational exposure

No data available

3.2 Clinical exposure

Patients with clinical signs of contact sensitivity to a therapeutically used triphenylmethane dye (gentian violet – which is structurally similar to malachite green, with an extra amine group) were patch tested with malachite green (form not specified) (2% in water). Positive patch-test reactions (recorded as isolated papules, oedema, confluent papules and infiltration) were observed in 6/11 patients, however, in many instances the erythematous reaction was obscured by the dye. This study suggests the possibility of cross-sensitisation between gentian violet and malachite green. (Bielicky and Novák, 1969).

The data available are not considered to be helpful for classification purposes

4. TOXICOLOGICAL DATA

4.1 ACUTE TOXICITY

4.1.1 Oral

Classification agreed by the Working Group in September 2002.

Species	LD_{50} (mg/kg)	Observations and remarks
Rat (sex not specified)	275	The substance tested was malachite green oxalate (> 90% purity). Acute effects observed included reduced motor activity, diarrhoea and hyperemia and atonia of the intestinal walls often in association with dilatation of the gastrointestinal tract (Clemmensen *et al*., 1984).
Rat (sex not specified)	520	The form of malachite green tested was not specified. Effects observed included depression, prostration, emaciation and coma (Meyer and Jorgenson, 1983).
The data available support the current classification of Xn; R22.		

4.1.2 Inhalation

No data available.

4.1.3 Dermal

Species	LD_{50} (mg/kg)	Observations and remarks
Rat 5/sex	>2000 (for a 20% suspension)	Animals were administered a 20% suspension of malachite green oxalate (2000 mg/kg) (> 90% purity) under an occlusive dressing (period of exposure not specified). No deaths or signs of systemic toxicity were observed (Clemmensen *et al*., 1984).
No classification justified		

4.1.4 Skin irritation

Species	No. of animals	Exposure time (h)	Conc. (w/w)	Dressing: (occlusive, semi-occlusive, open)	Observations and remarks (specify degree and nature of irritation and reversibility)
Rat	5/sex	Not specified	400µl of a 20% suspension	Occlusive	Limited information available from an acute dermal toxicity study suggests application of a 20% suspension of malachite green oxalate (2000 mg/kg) (> 90% purity) does not cause skin irritation (period of exposure not specified) (Clemmensen *et al*., 1984).
No classification justified					

4. TOXICOLOGICAL DATA (continued)

4.1.5 Eye irritation

Classification agreed by the Working Group in January 2003.

Species	No. of animals	Conc. (w/w)	Observations and remarks (specify degree and nature if irritation, any serious lesions, reversibility)
Rabbit	3	8%	In this poorly reported study, marked oedema, substantial discharge and slight hyperaemia of the conjunctiva were observed following instillation of 100µl (76 mg/kg) of an aqueous solution of malachite green oxalate (> 90% purity). These effects were no longer evident in 2/3 rabbits after 24 hours. No further details available (Clemmensen *et al*., 1984).
Rabbit	1	(Not stated)	In a single rabbit, instillation of fine malachite green oxalate crystals (particle size 60-90 µm) produced a totally opaque cornea and bright red and oedematous conjunctivae. This effect persisted for up to 14 days. No further details presented (Clemmensen *et al*., 1984).
The data available support the current classification of Xi; R41. See Annex for discussion.			

4.1.6 Irritation of respiratory tract

No data available.

4.1.7 Skin sensitisation

Species	Type of test	No. of animals	Incidence of reactions observed
Guinea pig	Maximisation	Not stated	In this poorly reported study, intra-dermal and topical induction was at 0.2 and 20% aqueous suspension of malachite green oxalate (> 90% purity), respectively. There was no response following challenge at up to 1% (Clemmensen *et al.*, 1984).
No classification justified			

4.2 REPEATED OR PROLONGED TOXICITY GROUPED ACCORDING TO SUBACUTE AND SUBCHRONIC TOXICITY

4.2.1 Oral

Species/ strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat (Wistar) 8/sex/group	0, 10, 100 or 1000 ppm in diet (approx 0, 1, 10 or 100 mg/kg bw/d)*	28 days	No clinical signs of toxicity were evident at 1 or 10mg/kg malachite green oxalate, but hyperactive behaviour was observed at 100 mg/kg. Animals in this group also showed a significant reduction in body weight gain and food consumption (not quantified). Slight haematological changes were noted in females at the highest dose. No further details available (Clemmensen *et al.*, 1984).

Rat (Fischer 344) 8/sex/dose group	0, 25, 100, 300, 600 and 1200 ppm in diet (approx 0, 2.5, 10, 30, 60 & 120 mg/kg bw/d)* Malachite green hydrochloride (≥ 94% purity) was tested.	28 days	Clinical signs of toxicity were limited to a decrease in mean body weight in females at the highest dose during weeks 1 to 4 (approx 80% of controls). The liver appeared to be the target organ with increased relative liver weight (top 2 doses in males and top 3 doses in females), a dose-related increase in the levels of γ-glutamyl transferase (4.2-fold greater than control in high dose females) and minimal to mild hepatocyte vacuolisation in 7/8 females at 120 mg/kg and 1/8 and 4/8 males at 60 and 120 mg/kg, respectively evident. Slight haematological changes of no toxicological significance were also noted (Culp *et al*., 1999). * The food intake or actual doses ingested were not presented. Approximate doses have been calculated by HSE using default values for both food intake and body weights. See Annex B.
Mice ($B6C3F_1$) 8/sex/dose group	0, 25, 100, 300, 600 & 1200 ppm in diet (approx 0, 5, 20, 60, 110 & 220 mg/kg bw/d)*.	28 days	Clinical signs of toxicity were limited to a decrease in body weight (approx 91% of controls) in females at the highest dose during weeks 3 and 4. Slight changes in haematological parameters were noted in both males and females but were not considered toxicologically significant. No significant histopathological changes were evident (Culp *et al.,* 1999). * The food intake or actual doses ingested were not presented. Approximate doses have been calculated by HSE using default values for both food intake and body weights. See Annex B.

Studies not previously considered by the group

Species/strain	**Dose (mg/kg bw, mg/kg diet)**	**Duration of treatment**	**Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)**
Rat (Fischer 344) 8/sex/dose group	0 and 1200 ppm in diet (approx 0 & 220 mg/kg bw/d)*. Malachite green hydrochloride (≥ 94% purity) was tested	4 or 21 days	Further details on the Culp study, over and above those presented to the group previously are now available and are presented below. Additional rats (8/sex/dose /time point) were fed 0 or 1200 ppm malachite green for 4 or 21 days then T3, T4 and TSH levels were measured. T3 levels were significantly increased in females at 21 days (105.4 and 123.1ng/dl for control and 1200ppm respectively), and T4 levels were significantly decreased in females at 4 and 21 days (4 days: 3.1 and 2.6 ug/dl for control and 1200ppm respectively, 21 days: 3.0 and 2.5 ug/dl for control and 1200ppm respectively). There were no significant changes in T3 and T4 levels for males and no significant changes in TSH for either sex (Culp *et al*., 1999).
No classification justified			

4.2.2 Inhalation

No data available.

4.2.3 Dermal

No data available.

4.3 CARCINOGENICITY (INCLUDING CHRONIC TOXICITY STUDIES)

Previous discussion regarding the carcinogenic potential of malachite green was postponed pending new information from NTP. This data is now available and presented below under 'New Data'.

4.3.1 Oral

4.3.1.1 Data presented previously

Species/strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
Rat (strain not specified) 10/sex/test & control groups respectively	0, 0.03, 0.3 & 3.0 % in diet (approximately 15, 130 & 1320 mg/kg bw/d)* The form of malachite green tested was not specified.	64 weeks	All rats within the two highest dose groups died within the first week of the study. Increased mortality was observed at the lowest dose in males by week 20 (3/10 deaths compared with 1/10 in controls). A significant decrease in body weight (80% of control) and food consumption (89% of control) was observed in low dose females by week 64. Observations were limited to a significant increase (110% of controls) in liver organ weight in females, and altered spermatogenesis in males at the lowest dose (1/3 rats compared with 1/5 in controls – non significant) (Allmark and Grice, 1957). * The food intake or actual doses ingested were not presented. Approximate doses have been calculated by HSE using default values for both food intake and body weights See Annex B.

4. TOXICOLOGICAL DATA (continued)

4.1.3.2 New data

Species/strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
Rat (F344) 48 female/ dose group	0, 100, 300 or 600ppm (approx. 0, 7, 21, and 43 mg/kg bw/day) NTP,2005 Malachite green chloride 87% pure	104 weeks	The carcinogenicity of malachite green was investigated in a dietary carcinogenicity study in female F344/N Nctr BR rats. Females were shown to be the most sensitive sex during a range finding study and hence were the only sex tested. There was no significant toxicity at any dose and survival was similar in all dose groups. Body weight gain was reduced in the top two doses (~10%). Relative liver weights were significantly increased in high dose female rats (35.70 and 41.06 mg organ weight/g body weight for control and 600ppm respectively). Non-neoplastic findings consisted of a dose related increasing trend of thyroid gland cystic follicles (0/46, 1/48, 1/47, 3/46, for control 100, 300, and 600ppm respectively) and an increase in eosinophilic foci in the liver (5/48, 10/48, 13/48, 14/48, for control 100, 300, and 600ppm respectively). There were no statistically significant increases in tumour incidences. However, historical controls incidences were exceeded for adenoma/ carcinoma (combined) of thyroid follicular cells at the top two doses (0/46(0%), 0/48(0%), 3/47(6%) and 2/46(4%) in control, 100, 300, and 600 dose groups, respectively –historical control 1.4%, range 0-3%), and mammary gland carcinoma at the top dose (2/48(4%), 2/48(4%), 1/48(2%), and 5/48(10%) in control 100, 300, and 600 groups, respectively - historical control 0.7%, range 0-4%). Minimal increases in hepatocellular adenomas were also observed (1/48(2%), 1/48(2%), 3/48(6%), and 4/48(8%) in control, 100, 300, and 600 dose groups, respectively - historical control 0.2%, range 0-0.6%). There was a decreased incidence in a dose–related trend of mononuclear cell leukaemia, which was significant in the top two doses (19/48(40%), 17/48(35%), 10/48(21%), 1/48(2%) in control, 100, 300, and 600 groups, respectively) (NTP, 2005).

Species/strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
Mouse (B6C3F1) 48 female/ dose group	0, 100, 225 or 450 ppm (approx. 0, 15, 33, 67 mg/kg bw/day) NTP2005 Malachite green chloride 87% pure	104 weeks	The carcinogenicity of malachite green was investigated in a dietary carcinogenicity study in female $B_6C_3F_1$/Nctr BR mice. Females were the most sensitive sex during a range finding study and hence were the only sex used. There was no significant toxicity at any dose and survival was similar in all dose groups. Body weight gain was reduced in the top dose in mice (5-10%). Relative kidney weights were less in dosed mice that that of the controls. Non- neoplastic findings consisted of increased incidences of intracytoplasmic inclusions of the urinary bladder (7/47, 15/46, 34/45, 39/48 for control 100, 300, and 600ppm respectively). There was no increase in tumour incidence in exposed mice (NTP, 2005).

4.3.2 Inhalation

No data available.

4.3.3 Dermal

No data available.

4.4 GENOTOXICITY

Previous discussion regarding the genotoxic potential of malachite green was postponed pending new information from NTP. This data is now available and presented below under 'New Data'.

On the basis of the data presented below, it is proposed that this substance be classified as a Category 3 Mutagen. See Annex for discussion.

4.4.1 *In vitro* studies

4.4.1.1 Data presented previously

Test	Cell type	Conc. range	Metabolic activation	Observations and remarks
Ames	*Salmonella typhimurium* TA 98, TA 100, TA 1535 and TA 1537	0.05-160 µg/plate	+/- S9	**Positive** in a well-conducted Ames test in strain TA 98 at 6.4, 32 and 160 µg/plate; only in the presence of S-9 (Clemmensen *et al.*, 1984). Malachite green oxalate tested (> 90% purity).
Ames	*Salmonella typhimurium* TA97a, TA98, TA100 and TA102	0.01-10 µg/plate	+/- S9	**Negative** in all strains in a well-conducted Ames test (Fessard *et al*., 1999). Malachite green oxalate tested (70.8% purity).
Mammalian cell gene mutation (*Hgprt*)	CHO	0.001-1µg/ml	+/- S9	**Negative.** In the absence of metabolic activation malachite green oxalate (70.8% purity) was highly toxic at doses greater than 0.1 µg/ml. No reproducible increase in the number of thioguanine-resistant mutants was observed at sub-cytotoxic concentrations in the presence or absence of S9 (Fessard *et al*., 1999).
Comet	CHO	1-20 µg/ml	+/- S9	**Positive.** Malachite green oxalate (70.8% purity) was shown to induce DNA damage in CHO cells following exposure for 1 hour at doses ≥ 3 µg/ml in the absence of S9. In the presence of S9, a significant increase in DNA damage was observed at 15 and 20 µg/ml with only a moderate associated decrease in cell viability (10-20%) (Fessard *et al*., 1999).
Chromosome aberration	CHL	4.0 mg/ml	-S9	**Positive.** Limited details available from a screening study indicate that malachite green causes a significant increase (28%) in the number of chromosomal aberrations in CHL cells at a harvest time of 48 hours. No information regarding the types of aberrations observed was presented (Ishidate, 1981). The form of malachite green tested was not stated.

Test	Cell type	Conc. range	Metabolic activation	Observations and remarks
Chromosome aberration	CHO	1-20 μm/plate	-S9	**Negative,** with limitations. Results were reported only for the top dose and no information on cytotoxicity was presented. There was a slight but non-statistically significant increase in the number of chromosome breaks (0.26 breaks per metaphase compared with 0.0-0.16 in controls) (Au and Hsu, 1979). The absence of information on cell viability and dose-response prevent any reliable conclusions from being drawn. The form of malachite green tested was not stated.

4.4.2 *In vivo* studies (somatic cells)

4.4.2.1 Data presented previously

Test	Species	Tissue	Sampling time	Observations and remarks
DNA adducts	Rat (F344) 8 male/group Mouse (B6C3Fl) 8 female/ group	Liver	28 days	**Positive.** Male rats and female mice received malachite green hydrochloride (0, 100 or 600 ppm) (≥ 94% purity) in the diet for 28 days. At the end of the treatment period, DNA was isolated from the livers, and adduct levels measured using ^{32}P-postlabelling with n-butanol enrichment. A single type of adduct (or co-eluting adducts) was observed in both rats and mice, with adduct levels increasing significantly as a function of the dose (Culp *et al*., 1999).
Micronucleus (OECD)	Mouse (NMRI) 5/group	Bone marrow	24, 42 & 66 h (75% of the LD_{50})	**Negative.** Mice were administered a single oral gavage dose of 37.5 mg/kg malachite green oxalate (> 90% purity). No significant increase in the incidence of micronuclei was observed at any sampling time. The positive control, cyclophosphamide, gave an appropriate response. The PCE/NCE ratio was not reported (Clemmensen *et al*., 1984).
Mammalian spot test	Mouse (C57B1/6J Han)	Melano blasts	Exposed on days 8, 9 & 10 of pregnancy	**Negative.** Limited details are available from an abstract. Mice were administered malachite green (10, 20 & 40 mg/kg) by gavage on days 8, 9 & 10 of gestation. No significant increase in the number of recessive spots was observed in the offspring. The positive control, ENU, gave an appropriate response (Jensen, 1984). The form of malachite green tested was not stated.

4. TOXICOLOGICAL DATA (continued)

4.4.2.2 New data

Test	Species	Tissue	Sampling time	Observations and remarks
Gene cell mutation assay in transgenic animals **(a)** *lacII* mutation assay	Mouse (B6C3F1) 12female /group 6 animals sacrificed/ group after 28 days 88% pure	Liver	28 days and 16 weeks	**Negative.** Female mice received malachite green chloride (0 or 450 ppm) in the diet for 16 weeks then 10ug DNA from each animal was extracted and analysed. Malachite Green did not increase the c*II* liver mutation frequency after 16 weeks.
(b) Lymphocyte mutation assay (*Hprt*)		Spleen		Female mice received malachite green chloride (0 or 450 ppm) in the diet for 28 days and 16 weeks. H*prt* lymphocyte mutant frequencies were not significantly different from controls after 28 days or 16 weeks (Mittelstaedt *et al*., 2004).
Micronucleus	Mouse (B6C3F1) 12female /group 6 animals sacrificed/ group after 28 days 88% pure	Peripheral blood erythrocytes	28 days and 16 weeks	**Negative.** Female mice received malachite green chloride (0 or 450ppm) in the diet for 28 days and 16 weeks then 100ul blood was sampled for mutations. No effect on reticulocyte or normochromatic erythrocyte peripheral blood micronucleus frequencies was observed. PCE/NCE ratios were: (%) 4 weeks 0.11 and 0.13; 16 weeks 0.11 and 0.12 for controls and 450ppm respectively. Positive and negative controls gave expected results (Mittelstaedt *et al*., 2004).

4.5 FERTILITY

No data available.

4.6 DEVELOPMENTAL TOXICITY

The first study presented below (in rabbits) has been summarised previously by the UK in document ECBI/54/02. More detailed information on this study is presented below.
The Working Group has previously seen the teratology studies in the rat (Reynolds 2001 and 2002), and they are summarised here for the sake of completeness. As the WG is already aware of the information no change in classification is proposed. Classification was agreed by the Working Group in January 2003.

Species	Route	Dose	Exposure time	Observations and remarks

Rabbit (New Zealand) 33/dose group Further details	Oral	0, 5, 10 & 20 mg/kg Malachite green oxalate	Days 6-18 of gestation. Sacrificed on day 29.	An increased incidence of mortality was observed in all treated groups (12/33, 5/33 and 1/33 at 5, 10 and 20 mg/kg, respectively). The authors attributed these deaths to acute pulmonary toxicity resulting from aspiration of malachite green into the lungs during the dosing procedure and therefore were not treatment related. No other overt signs of toxicity were evident during the study. A reduction in mean maternal body weight relative to control was observed at the two highest doses, however this effect was only statistically significant at 10 mg/kg and not clearly related to dose. There was a dose-related increase in the mean number of resorptions per animal (0.8, 2.1, 2.3 and 3.8 at 0, 5, 10 and 20 mg/kg, respectively). A statistically significant increase in postimplantation loss (15%, 35%, 34% and 42% at 0, 5, 10, and 20 mg/kg, respectively). . Foetal body weights were reduced at all doses, (92%, 96% & 95% of control at 5, 10 & 20 mg/kg, respectively). Developmental anomalies (gross, visceral, and skeletal) were observed at all treatment levels but were not dose-related (18%, 38%, 34% & 47% at 0, 5, 10 & 20 mg/kg, respectively). Visceral abnormalities observed in foetuses included enlarged liver and heart. Skeletal abnormalities evident included; incomplete ossification of the skull, malformed skull, twisted ankles, shortened tail and malformed scapula (Meyer and Jorgenson, 1983).

Species	**Route**	**Dose**	**Exposure time**	**Observations and remarks**
Rat (CD) 6/dose group	Oral	0, 10, 30, 100 mg/kg Malachite green oxalate	Days 6-15 gestation. Sacrificed on day 20	Conducted as a range finding study. Malachite green oxalate was administered by gavage to CD rats on day 6-15 gestation, which were then sacrificed on day 20. One female in 100mg/kg dose group was killed in extremis on day 12 post coitum. Green staining of the GI tract was apparent on necroscopy. Decreased body weight gain and food consumption and an increase in water intake also occurred in the dams in the top dose group. No treatment related macroscopic changes occurred in the dams or pups. Litter size, survival in utero and mean foetal and placental weights were unaffected by treatment. No further details available (Reynolds 2001).

Species	**Route**	**Dose**	**Exposure time**	**Observations and remarks**
Rat (CD) 22/dose group	Oral	0, 2, 10, 50 mg/kg and separately 0 and 100 mg/kg Malachite green oxalate	Days 6-15 gestation. Sacrificed on day 20	Malachite green oxalate was administered by gavage to CD rats on day 6-15 gestation, which were then sacrificed on day 20. Five females in 100mg/kg dose group were killed in extremis. Green/blue tinged salivary glands and blue staining of the GI tract were apparent on necroscopy. Decreased body weight gain and food consumption and an increase in drinking water intake also occurred in the dams in the top dose group. No treatment related macroscopic changes occurred in the dams or pups. Litter size, survival in utero and mean foetal and placental weights were unaffected by treatment. No further details available (Reynolds 2002).

REFERENCES

Allmark, M. G. and Grice, H. C. (1957): Chronic toxicity studies on food colours. *J. Pharm. Pharmacol.,* **9**: 622-628.

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

Bielicky, T and Novák, M. (1969): Contact group sensitisation to triphenylmethane dyes. *Arch Derm.,* **100**: 540-543.

Clemmensen, S., Jensen, J. C., Jensen, N. J., Meyer, O., Olsen, P. and Würtzen, G. (1984): Toxicological studies on malachite green: a triphenylmethane dye. *Archives of Toxicology.* **56**: 43-45.

Culp, S. J., Blankenship, L. R., Kusewitt, D. F., Doerge, D. R., Mulligan, L. T. and Beland, F. A. (1999): Toxicity and metabolism of malachite green and leucomalachite green during short-term feeding to Fischer 344 rats and B6C3F_1 mice. *Chemico-Biological Interactions.,* **122**: 153-170.

Fessard, V., Godard, T., Huet, S., Mourot, A., and Poul, J. M. (1999): Mutagenicity of malachite green and leucomalachite green in *in vitro* tests. *Journal of Applied Toxicology.* **19**: 421-430.

Ishidate, M. (1981): Application of chromosome aberration test in vitro to the primary screening for chemicals with carcinogenic and/or genetic hazards. Tests Courts Cancerog. Quo Vadis Symposium 57-79.

Jensen, N. J. (1984): Lack of mutagenic activity of malachite green in the mammalian spot test. *Mutat Res.,* **130**: 248.

Meyer, F. P. and Jorgenson, T. A. (1983): Teratological and other effects of malachite green on development of rainbow trout and rabbits. *Transactions of the American Fisheries Society.,* **112**: 818-824.

Mittelstaedt, R. A., Mei, N., Webb, P. J., Shaddocj, J. G., Dobrovolsky, V. N., McGarrity, L. J., Morris, S. M., Chen, F. A., Greenlees, K. J., Heflich, R. H. (2004): Genotoxicity of malachite green and leucomalachite green in female Big Blue B6C3F1 mice. *Mutat. Res.* **561(1-2)**: 127-38.

NTP Technical Report TR-527 Toxicology and Carcinogenesis Studies of Malachite Green and Leucomalachite Green in F344 rats and B6C3F1 mice (feed studies) NTP US Dept Health and Human Services (2005)

Reynolds, S. M. (1991): Astrazon Grun M: Preliminary teratology study in the rat. Final report. *Life Science Research (LSR)* Report 91/0296.

Reynolds, S. M. (1992): Astrazon Grun M: Teratology studies in the rat. Final report. *Life Science Research (LSR)* Report 92/0056.

Annex A:

EC CLASSIFICATION AND LABELLING: MALACHITE GREEN

Endpoints discussed and agreed previously.

Acute toxicity

Rat oral LD_{50} values of 275 and 520 mg/kg were gained, supporting the current classification of **Xn; R22.**

Skin irritation

A poorly reported study demonstrated that a 20% suspension of malachite green did not cause any signs of skin irritation. There is currently no evidence to inform on whether higher concentrations would cause irritation. Agreed for **no classification**.

Eye irritation

The eye irritant potential of an aqueous solution and solid form of malachite green has been investigated.

In a guideline study in rabbits, an 8% aqueous solution of malachite green produced marked oedema, substantial discharge and slight hyperaemia of the conjunctiva, which was shown to be reversible after 24 hours in 2 out of 3 animals.

In a second poorly reported study from the same laboratory, treatment of a single rabbit with fine malachite green crystals produced a totally opaque cornea and bright red and oedematous conjunctivae, which persisted throughout the observation period (14 days).

The severity and persistence of the effects observed are sufficient to support the current classification: **Xi; R41**.

Repeated dose toxicity

The repeated-dose toxicity of malachite green has been investigated in two 28-day studies in rats and one study in mice. The main findings in both species were limited to effects in the liver including; an increase in relative liver weight and minimal to mild hepatocyte vacuolisation. Changes in T3 and T4 levels in the thyroid also occurred in rats. These findings do not indicate significant toxicity following repeated dosing at doses relevant for classification. Agreed for **no classification**.

Reproductive toxicity

Fertility: No data available. Agreed for **no classification.**

Developmental toxicity: The full study report details of a rabbit teratogenicity study together with data from a preliminary and main teratogenicity study in the rat are available.

The developmental toxicity of malachite green has been investigated in two species, rat and

rabbit. No evidence of developmental toxicity was evident in rats at dose levels causing maternal toxicity (increased mortality and reduced bodyweight). An older study conducted in rabbits provides some indication of possible developmental toxicity, evidenced by an increase in the number of resorptions at doses that did not cause significant maternal toxicity. However, the poor quality of the study and concerns relating to its conduct cast some doubt on the reliability of the findings.

Overall, there are inconsistent findings in the rat and rabbit. The findings from the rabbit study provide evidence that malachite green may cause developmental toxicity and therefore classification is justified, supporting the current classification **Repr. Cat. 3; R63**.

Endpoints for discussion.

Mutagenicity

The genotoxicity of malachite green has been investigated in a number of studies. Malachite green gave somewhat contradictory findings in *in vitro* tests with positive findings in a number of standard *in vitro* mutagenicity tests (Ames, COMET, chromosomal aberration), and negative findings in others (mammalian cell gene mutation assay (*hgprt*) (+/-S9), and a chromosomal aberration test in CHO cells (-S9)).

In vivo malachite green tested negative in a number of standard mutagenicity tests (mouse micronucleus, mouse spot test following oral administration), and a mammalian gene mutation assay following oral administration, although it was shown to form DNA adducts in rats and mice following repeated dietary exposure.

Overall, there is clear evidence of genotoxicity *in vitro*. There is no direct evidence from the available *in vivo* studies that this genotoxicity is expressed *in vivo*. However, the observation of DNA adducts in the liver of rats and mice indicate that malachite green can reach and covalently bind to DNA, which could potentially lead to mutations. In view of this it would be prudent to presume that malachite green may be a potential *in vivo* somatic cell mutagen and therefore classification with **Muta. Cat. 3; R68** is proposed for discussion.

Carcinogenicity

The carcinogenicity of malachite green by the oral route has been investigated in a good quality study in rats and mice.

In female rats, there were no statistically significant increases in tumour incidence, although there were increased incidences of thyroid follicular cell adenoma and carcinoma combined (above historical controls at the top two doses), hepatocellular adenoma (above historical controls in all dose groups), and mammary gland carcinoma (above historical controls at the top dose group). Although the incidence of these tumours was increased above historical control levels the increases were relatively small and are not considered to provide reliable or convincing evidence of carcinogenicity. There were no tumour findings in female B6C3F1 mice. It is noted that malachite green is genotoxic, but the clearly negative carcinogenicity findings suggest that this genotoxic activity does not contribute to or facilitate a carcinogenic process. Overall, it is considered that the evidence is not sufficient to warrant classification for carcinogenicity. **No classification** is proposed for discussion.

Annex B: Calculation of ingested dose from dietary studies

Where dietary studies did not present actual doses received, the ingested dose, in terms of mg/kg/day, was estimated using the following default values.

Species	Sex	Bodyweight (Kg)	Food intake (g/day)
Rat (lifetime studies)	Male Female	0.5 0.35	20 17.5
Rat (short term studies)	Male Female	0.2 0.175	20 17.5
Mouse	Male Female	0.03 0.025	5 5

References

Gold *et al*., (1984). *Environ. Health. Persp.*, **58**, 9-319.

10.2 ANNEX II

HUMAN HEALTH CLASSIFICATION AND LABELLING: LEUCOMALACHITE GREEN

FORM XI/396/93

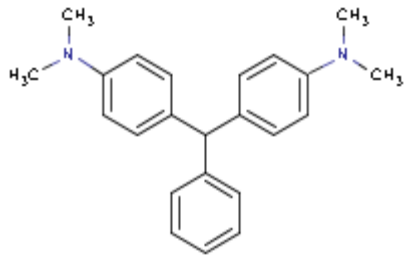
Commission of the European Communities DG XI

CLASSIFICATION AND LABELLING OF DANGEROUS SUBSTANCES
Recommended form to be used for the proposed classification and labelling of Dangerous Substances in order to update Annex 1 of Directive 67/548/EEC

Date: June 2005	Prepared by: Health and Safety Executive, UK

The information contained in this form is not regarded as confidential

1. IDENTIFICATION OF THE SUBSTANCE

INDEX No. None	**EC No. 204-961-9 CAS No. 129-73-7 ID No.**
1.1 EINECS Name **If not in EINECS IUPAC Name**	**10.3 N,N,N',N'-TETRAMETHYL-4,4'-BENZYLIDENEDIANILINE**
1.2 Synonyms (state ISO name if available)	Leucomalachite Green
1.3 Molecular formula	$C_{23}H_{26}N_2$
1.4 Structural formula	
1.5 Purity (w/w)	
1.6 Significant impurities or additives, their concentrations (w/w)	No data available
1.7 Known uses	**Industrial:** Histological stain. **General public:**
1.8 Proposed classification	**Carc. Cat. 3; R40 : Muta. Cat 3; R68 : Repr. Cat. 3; R63 :Xn, R22 : Xi; 41**
1.9 Proposed label **Symbol**:	**R phrase(s):** R22-40-41-63-68 **S phrase(s):** (2-) 26-36/37/39 (-46) (-60-61) Xn

EXISTING LABEL	In Annex 1: No

2. PHYSICO-CHEMICAL CHARACTERISTICS

2.1 Physical form	Faint green solid
2.2 Molecular weight	330
2.3 Melting point/range (^{o}C)	
2.4 Boiling point/range (^{o}C)	
2.5 Decomposition temperature	
2.6 Vapour pressure (Pa(^{o}C))	
2.7 Relative density (g/ml)	
2.8 Vapour density (air = 1)	
2.9 Fat solubility (mg/kg, ^{o}C)	
2.10 Water solubility (mg/kg, ^{o}C)	
2.11 Partition coefficient (log Pow)	
2.12 Flammability **flash point (^{o}C)** **explosivity limits (%,v/v)** **auto-flammability temp. (^{o}C)**	 **open cup:** **closed cup:** **lower limit:** **upper limit:**
2.13 Explosivity **danger of explosion as a result of:** **explosive properties at high temperature**	No data available **shock:** **friction:** **ignition:**
2.14 Oxidising properties	No data available
2.15 Other physico-chemical properties **(eg. liberates toxic gas on heating or in contact with water or acids)**	No data available

3. OBSERVATIONS ON HUMANS

Where available, human data are considered to be of more relevance in determining the potential effects of chemical substances on the human population. (Annex V, Directive 67/548/EEC).
3.1 Occupational exposure No data available
3.2 Clinical exposure No data available

4. TOXICOLOGICAL DATA

4.1 ACUTE TOXICITY

4.1.1 Oral

No data available.

4.1.2 Inhalation

No data available.

4.1.3 Dermal

No data available.

4.1.4 Skin irritation

No data available.

4.1.5 Eye irritation

No data available.

4.1.6 Irritation of respiratory tract

No data available.

4.1.7 Skin sensitisation

No data available.

4.2 REPEATED OR PROLONGED TOXICITY GROUPED ACCORDING TO SUBACUTE AND SUBCHRONIC TOXICITY

4.2.1 Oral

Species/strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, NOAEL, effects of major toxicological significance)
Rat (Fischer 344) 8 male/dose group	0, 290, 580, and 1160 ppm in diet Leucomalachite green ≥ 98% purity	28 days	Further details on the Culp study, detailing leucomalachite green (previously only malachite green results presented) are now available and are presented below. There were no significant clinical signs of toxicity (<10% bodyweight loss at the highest dose). The liver appeared to be the target organ with significantly increased relative liver weight at all 3 dose levels, an increase in the levels of γ-glutamyl transferase in the top dose group (2.2-fold greater than control, P<0.05), and slight increases in phosphorous levels in the top dose group (10% increase P<0.05). A significant dose-related trend in hepatocyte vacuolisation was observed (2/8, 5/8, 7/8 for 290, 580 and 1160ppm dose groups respectively). Slight, but significant haematological changes were noted in the top dose group. Apoptotic follicular epithelial cells in the thyroid gland were observed at the top two doses (2/8 in 580ppm and 2/8 in 1160ppm). Sloughed follicular cells with condensed nuclei located within the follicles were observed. There was no inflammatory reaction, and there was evidence of follicular epithelium regeneration (Culp *et al*., 1999).
Rat (Fischer 344) 8 male/dose group	0 and 1160ppm in diet	4 or 21 days	Additional rats (8/dose /time point) were fed 0 or 1160ppm leucomalachite green for 4 or 21 days, then T3, T4 and TSH levels were measured. A significant decrease in T4 levels and a significant increase in TSH levels was found at 4 and 21 days (T4 4 days: 5.0 and 3.4ug/dl for control and 1160ppm respectively, 21 days: 3.0 and 2.3ug/dl for control and 1160ppm respectively. TSH 4 days: 1.9 and 3.0ng/ml for control and 1160ppm, 21 days: 3.7 and 6.3 ng/ml for control and 1160ppm (Culp *et al*., 1999).
Mouse ($B6C3F_1$) 8 female/dose group	0, 290, 580, and 1160 ppm in diet ≥ 98% purity	28 days	There were no significant clinical signs of toxicity (<10% bodyweight loss at the highest dose). All mice in the top dose group had scattered dead or degenerate cells in the transitional epithelium of the urinary bladder (Culp *et al*., 1999).
No classification justified			

4.2.2 Inhalation

No data available.

4.2.3 Dermal

No data available

4.3 CARCINOGENICITY (INCLUDING CHRONIC TOXICITY STUDIES)

On the basis of the data presented below, it is proposed that this substance be classified as a Category 3 Carcinogen. See Annex for discussion.

4.3.1 Oral

Species/ strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
Rat (F344) 48/sex/ dose group	0, 91, 272 or 543 ppm (approx. 0, 5, 15, and 30 mg/kg bw/day males; 0, 6, 17, 35 mg/kg bw/day females)	104 weeks	The carcinogenicity of leucomalachite green was investigated in a standard dietary carcinogenicity study in male and female F344/N Nctr BR rats. Survival of 272ppm males was greater than that of controls. Mean body weights of 543 and 272ppm males and 272 and 91ppm females were less than that of controls throughout the study. Relative liver weights were significantly increased in 272 and 543ppm males and females (males: 34.30, 43.55, 51.69 mg organ weight/g body weight for control, 272 and 543ppm respectively; female: 33.76, 37.87, 46.57 mg organ weight/g body weight for control, 272 and 543ppm respectively). Relative thyroid gland weights of 543ppm males (0.10 and 0.11 mg organ weight/g body weight for control and 543ppm groups respectively) and females (0.11 and 0.14mg organ weight/g body weight for control and 543ppm respectively) were significantly increased. Non-neoplastic findings consisted of an increasing trend of thyroid gland cystic follicles in males and females (males: 0/47, 0/47, 0/48, 3/46 for control 91, 272, or 543ppm respectively; female: 0/46, 1/46, 0/47, 2/48 for control, 91, 272, or 543ppm respectively) and an increase in eosinophilic foci in the liver (males: 3/48, 14/47, 19/48, 33/47 for control 91, 272, or 543ppm respectively; female: 3/48, 12/48, 20/48, 16/48 for control, 91, 272, or 543ppm respectively). Cystic degeneration was observed in male livers (4/48, 18/47, 13/48, 19/47 for control, 91, 272 and 543ppm respectively), and cytoplasmic vacuolization of the liver was observed in females (5/48, 5/48, 17/48, 22/48 for control, 91, 272, and 543ppm respectively). Hepatocellular adenomas were minimally increased in all male dose groups, and exceeded historical control ranges in males at 272ppm and female rats in the 91ppm and 543ppm dose groups. Incidences were: males 2/48(4%), 2/47(4%), 3/48(6%), 2/47(4%): females 1/48(2%), 3/48(6%), 0/48(0%), 3/48(6%) for control 91, 272 or 543 ppm respectively - not statistically significant. Historical control incidences for males are 0.7%, range 0-2%; females 0.2%, range 0-1%. Thyroid gland follicular cell adenomas or carcinomas (combined) and cysts were observed in males and females, and exceeded historical control ranges at the 543 and 91ppm group for males and the 272ppm group for females. Incidences were: males 0/47(0%), 2/47(4%), 1/48(2%), 3/46(7%) for control 91, 272 or 543ppm respectively - not statistically significant; females 0/46(0%), 1/46(2%), 2/47(4%), 1/48(2%), for control 91, 272 or 543ppm respectively - not statistically significant. Historical control incidences for males are 0.4%, range 0-2%; females 1.4%, range 0-3%. (NTP, 2005)

Species/ strain	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (specify group size, effects of major toxicological significance)
			An increasing trend in the combined incidence of mammary gland adenoma and carcinoma in female rats was observed. However, female body weight was reduced throughout the study and thus compounded the statistical power to detect treatment related increases, thus the NTP recommend these be discounted. Testicular interstitial (Leydig) cell adenoma occurred with a positive trend in males and was significantly increased in the top dose group (37/48(77%), 42/47(89%), 43/48(90%), 45/47(96%) for control, 91, 272 or 543 ppm respectively, historical control 85.7% range 69-90%; bilateral interstitial cell adenoma 22/48, 30/47, 38/48, 39/47 for control, 91, 272 or 543 ppm respectively). Incidences of mononuclear cell leukaemia were decreased in rats (males: 29/48(60%), 16/47(34%), 19/48(40%), 7/47(15%) for control, 91, 272 or 543ppm respectively, female: 17/48(35%), 8/48(17%), 5/48(10%), 8/48(17%) for control, 91, 272 or 543ppm respectively) and incidences of pituitary gland adenoma were significantly decreased in exposed male rats (30/45(67%), 19/46(41%), 21/48(44%), 13/45(29%) for control, 91, 272 or 543ppm respectively) (NTP, 2005).
Mouse (B6C3F1) 48 female/ dose group	0, 91, 204, 408 ppm (approx. 0,13, 31, 63 mg/kg bw/day) NTP2005	104 weeks	The carcinogenicity of leucomalachite green was investigated in a dietary carcinogenicity study in female $B_6C_3F_1$/Nctr BR mice. Female mice were used because they were more sensitive in a range finding study. Survival, mean body weights, and feed consumption were similar to that of controls. Relative kidney weights were significantly decreased in all dose groups. Non-neoplastic findings consisted of increased incidences of intracytoplasmic inclusions of the urinary bladder (14/46, 33/48, 44/47, 44/44 for 0, 91, 204 and 408ppm respectively). The incidence of hepatocellular adenoma or carcinoma (combined) occurred with a positive trend and the incidence was significantly increased in 408ppm mice (3/47(6%), 6/48(13%), 6/47(13%), 11/47(23%) for control, 91, 204, or 408ppm respectively, historical control incidences: 6%, range 0-11%). The incidences of hepatocellular adenoma were increased although were not statistically significant (3/47(6%), 6/48(13%), 5/47(11%), 9/47(19%) for control, 91, 204 or 408ppm respectively, historical control incidences: 4.6%, range 0-11%) (NTP2005).

4.3.2 Inhalation

No data available.

4.3.3 Dermal

No data available.

4.4 GENOTOXICITY

On the basis of the data presented below, it is proposed that this substance be classified as a Category 3 Mutagen. See Annex for discussion.

4.4.1 *In vitro* studies

Test	Cell type	Conc. range	Metabolic activation	Observations and remarks
Ames	*Salmonella typhimurium* TA97a, TA98, TA100 and TA102	10-2000 µg/plate	+/- S9	**Negative** in all strains in a well-conducted Ames test (Fessard *et al*., 1999).
Mammalian cell gene mutation (*Hgprt*)	CHO	5-100 µg/ml	+/- S9	**Negative.** In the absence of metabolic activation mutant frequencies were repeatedly above control values at 75ug/ml. In the presence of metabolic activation the mutant frequency at 5ug/ml was significantly increased in one experiment. Overall results indicate a negative result (Fessard *et al*., 1999).
Comet	CHO	5-500 µg/ml	+/- S9	**Negative.** Leucomalachite green had no significant effect on cell viability and DNA in the absence (5-500 ug/ml) and presence (25-300 ug/ml) of exogenous activation.(10-20%) (Fessard *et al*., 1999).

4.4.2 *In vivo* studies (somatic cells)

Test	Species	Tissue	Sampling time	Observations and remarks
Gene mutation assays in transgenic animals **(a)** *lacII* mutation assay	Mouse (Big Blue B6C3F1) 6 female/ group 6 animals sacrificed/ group after 28 days	Liver	16 weeks	**Positive.** Mice were fed 0 or 408ppm leucomalachite green for 16 weeks, then 10ug DNA from each animal was extracted and analysed. The degree of mutant independence for control and treated mice was similar. When *lacII* mutant frequencies were corrected for independence leucomalachite green significantly increased the incidences of liver *lacII* mutations, specifically G→T and A→T transversions.
(b) Lymphocyte mutation assay *(Hprt)*		Spleen	4 and 16 weeks	Female mice were dosed with 0, 204ppm or 408ppm leucomalachite green. At 4 weeks there was a significant difference among groups due to a relatively low mutant frequency in mice treated with 204ppm leucomalachite green. No significant difference was observed between mutant frequencies in any treated group or control when analysed via Dunnets test.

				At 16 weeks *Hprt* lymphocyte mutant frequencies were not significantly different from controls (Mittelstaedt *et al* 2004).

Test	Species	Tissue	Sampling time	Observations and remarks
Gene mutation assays in transgenic animals **(a)** *lac* I mutation assay	Rat (Big Blue) 6 female/ group	Liver	4, 16, 32 weeks	**Equivocal.** Doses of 0, 9, 27, 91, 272 or 543 ppm leucomalachite green was fed to female Big Blue rats for 4, 16 or 32 weeks. Lower dose groups were not analysed because there was no increase in mutant frequencies in the 91ppm or 272ppm groups. An approximately 3-fold increase in the *lac* I mutant frequency was found in the livers of rats fed 543ppm leucomalachite green for 16 weeks. No other significant differences were noted at any other dose or time point, indicating uncertainty over the significance of this increase (Culp *et al*., 2002). 80 mutants from the 16 week 543 ppm group had the 1080bp *lac* I gene sequenced. The liver lac I mutation frequency, when corrected for clonality was $36x10^{-6}$ and was not significantly different from the control frequency. The predominant mutation was G:C→A:T transitions (Majanatha *et al*., 2004).
(b) Lymphocyte mutation Assay (*Hprt*)		Spleen		Female rats were fed 0, 9, 27, 91, 272 or 543ppm leucomalachite green for up to 32 weeks. None of the doses or time points showed a significant increase in *Hprt* mutants over the appropriate control (control group lymphocyte mutant frequencies ranged from $3x10^{-6}$ to $12x10^{-6}$, leucomalachite green fed groups ranged from $2x10^{-6}$ to $11x10^{-6}$) (Majanatha *et al*., 2004).
DNA adducts	Rat (F344) 8male/group Mouse (B6C3Fl) 8 female/ group ≥ 98% purity	Liver	28 days	**Positive.** Male rats and female mice received leucomalachite green (0, 96, or 580ppm) in the diet for 28 days. At the end of the treatment period, DNA was isolated from the livers, and adduct levels measured using ^{32}P-postlabelling with n-butanol enrichment. A single type of adduct (or co-eluting adducts) was observed in both species (although only low levels observed in mice), with adduct levels increasing significantly as a function of the dose. (Culp *et al*., 1999).
DNA adducts	Rat (Big blue) 4 female/ group 98% purity	Liver	28 days	**Positive.** Female rats received leucomalachite green (0, 9, 27, 91, 272 or, 543ppm) in the diet for 28 days. At the end of the treatment period, DNA was isolated from the livers, and adduct levels measured using ^{32}P-postlabelling with n-butanol enrichment. An increase in liver DNA adduct (or co-eluting adduct) levels was observed from 92 ppm upwards. No discernable adduct was apparent in the 0, 9, or 27ppm dose groups (Culp *et al*., 2002).

Test	Species	Tissue	Sampling time	Observations and remarks
Micronucleus	Rat (Big Blue) 6 female/ group) 98% purity	Bone marrow	4, 16 and 32 weeks	**Negative.** Female rats were fed 0, 9, 27, 91, 272 or 543ppm leucomalachite green for up to 32 weeks. No significant increase in the incidence of micronuclei was observed at any sampling time. (Majanatha *et al*., 2004).
Micronucleus	Mouse (B6C3F1) 12 female/ group 6 animals sacrificed/ group after 28 days	Peripheral blood erythrocytes	4 and 16 weeks	**Negative.** Female mice were dosed with 0, 204ppm or 408ppm leucomalachite green, then 100ul blood was sampled for mutations. No effect on reticulocyte or normochromatic erythrocyte peripheral blood micronucleus frequencies was observed. PCE/NCE (%) for 4 weeks: 0.11!0.01, 0.11!0.01 and 0.11!0.00 for control, 204 and 408ppm respectively, 16 weeks: 0.11!0.00, 0.12!0.01 and 0.11!0.00 for control, 204 and 408ppm respectively. Positive and negative controls gave expected results (Mittelstaedt *et al* 2004).

4.5 FERTILITY

No data available.

4.6 DEVELOPMENTAL TOXICITY

No data available.

REFERENCES

Culp, S. J., Blankenship, L. R., Kusewitt, D. F., Doerge, D. R., Mulligan, L. T. and Beland, F. A. (1999): Toxicity and metabolism of malachite green and leucomalachite green during short-term feeding to Fischer 344 rats and B6C3F$_1$ mice. *Chemico-Biological Interactions.*, **122**: 153-170.

Culp, S. J., Beland, F. A., Heflich, R. H., Benson, R. W., Blankenshio, L. R., Webb, P. J., Mellick, P. W., Trotter, R. W., Shelton, S. D., Greenlees, K. J., Manjanatha, M. G. (2002): Mutagenicity and carcinogenicity in relation to DNA adduct formation in rats fed leucomalachite green. *Mutat. Res*, **506-507**: 55-63..

Fessard, V., Godard, T., Huet, S., Mourot, A., and Poul, J. M. (1999): Mutagenicity of malachite green and leucomalachite green in *in vitro* tests. *Journal of Applied Toxicology*. **19**: 421-430.

Manjanatha, M. G., Shelton, S. D., Bishop, M., Shaddock, J. G., Dobrovolsky, V. N., Heflich, R. H., Webb, P. J., Blankenship, L. R., Bland, F. A., Greenlees, K. J., Culp, S. J. (2004): Analysis of mutations and bone marrow micronuclei in Big Blue rats fed leucomalachite green. *Mutat. Res.* **547(1-2)**: 5-18.

Mittelstaedt, R. A., Mei, N., Webb, P. J., Shaddocj, J. G., Dobrovolsky, V. N., McGarrity, L. J., Morris, S. M., Chen, F. A., Greenlees, K. J., Heflich, R. H. (2004): Genotoxicity of malachite green and leucomalachite green in female Big Blue B6C3F1 mice. *Mutat. Res.* **561(1-2)**: 127-38.

NTP Technical Report TR-527 Toxicology and Carcinogenesis Studies of Malachite Green and Leucomalachite Green in F344 rats and B6C3F1 mice (feed studies) NTP US Dept Health and Human Services (2005)

Annex A : EC CLASSIFICATION AND LABELLING: LEUCOMALACHITE GREEN

The toxicity of leucomalachite green has not been studied in detail, with most of the available information relating to mutagenicity and carcinogenicity. To inform on other toxicological endpoints that have not been investigated it is considered useful to consider the toxicity of malachite green. The two chemicals are structurally very similar and may be expected to have similar physico-chemical properties. Comparison of the findings from repeated-dose and carcinogenicity studies also indicates similar toxicological activity of the two chemicals. Considering this, it is suggested that it may be appropriate to classify leucomalachite green in the same way as malachite green for those endpoints on which there are no data on the grounds of read-across. **Xn; R22 : Xi; R41 : Repr.Cat.3; R63** should be discussed.

Repeated dose toxicity

The repeated-dose toxicity of leucomalachite green has been investigated in a 28day study in rats and mice. The main findings in both species were effects in the liver and thyroid, including; an increase in relative liver weight and minimal to mild hepatocyte vacuolisation and apoptotic follicular epithelial cells in the thyroid as well as decreases in T4 levels and increases in TSH levels. These findings do not indicate significant toxicity following repeated dosing at doses relevant for classification. **No classification is proposed.**

11 MUTAGENICITY

The genotoxicity of leucomalachite green has been investigated in a number of studies, some of which are non-standard tests, including a study in transgenic animals.

Leucomalachite green tested negative in a number of standard *in vitro* (Ames test, COMET assay in CHO cells, and in a mammalian cell gene mutation assay (*Hgprt*) (all +/-S9)) and *in vivo* (two mouse micronucleus tests *in vivo* in bone marrow and blood erythrocytes following oral administration).

One gene mutation test in transgenic animals was positive (based upon liver *lacII* gene mutations), and a second gave equivocal results (based upon liver *lacI* gene mutations).
^{32}P-post-labelling studies in rats and mice exposed for 28 days in the diet demonstrated the formation of DNA adducts in the liver, thus indicating leucomalachite green's ability to covalently bind to DNA.

The findings from standard mutagenic tests do not indicate any mutagenic activity. However, mutations in genes in the liver of transgenic mice and DNA adducts in the liver of rats and mice indicate that leucomalachite green can reach and covalently bind to DNA, and can cause mutations in this organ.

In view of these findings it is considered prudent to presume that leucomalachite green is a potential *in vivo* somatic cell mutagen and therefore classification with **Muta Cat 3; R68** is proposed for discussion.

Carcinogenicity

The carcinogenicity of leucomalachite green by the oral route has been investigated in a good quality study in mice and rats.

The evidence of possible carcinogenicity was a statistically significant dose-related increase in hepatocellular adenoma or carcinoma (combined) in female mice, the incidence of which exceeded historical control ranges. In rats, there were no statistically significant increases in tumour incidence, although the incidence of hepatocellular adenoma and thyroid gland follicular cell adenoma or carcinoma was increased in both sexes and some incidences were above historical controls. Mechanistic studies have shown that leucomalachite green inhibits thyroid peroxidase suggesting that the thyroid tumours were induced by perturbation of thyroid hormone homeostasis. There was also an increase in interstitial (Leydig) cell adenoma of the testes occurring with a positive trend was observed in F344 rats (statistically significant in the top dose group), but Leydig cell tumours in this strain of rat are not considered to be relevant for humans.

The evidence for carcinogenicity is not substantial, with limited evidence of tumour induction in the liver in mice (in a strain generally regarded as being particularly sensitive to the induction of such tumours) and only equivocal evidence of induction of liver tumours in female rats. It is recognised that this is only weak evidence for carcinogenicity, and the tumour profile is not typical for a genotoxic agent, but the statistically significant induction of tumours, with genotoxicity possibly involved in their induction, does raise some concern for carcinogenicity. Overall, classification with **Carc.Cat.3; R40** is considered justified and is proposed for discussion.

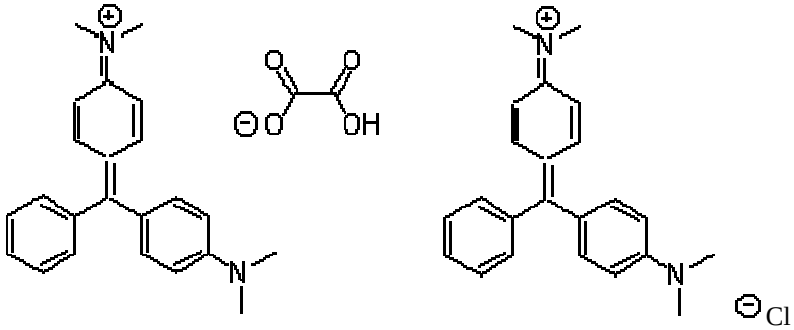
10.3 ANNEX III

This Annex contains exactly the same information seen by the TC C&L leading to a classification decision as N; R50-53.

ENVIRONMENTAL CLASSIFICATION AND LABELLING: LEUCOMALACHITE GREEN

UK environmental proposal: R50-53 by read across to Malachite Green

Supporting information: structural data for Malachite Green

INDEX No. None	A: **EC No.** 209-322-8 **CAS No.** 569-64-2 **ID No.** 602-096-00-5 B: **EC No.** 219-441-7 **CAS No.** 2437-29-8
EINECS Name **If not in EINECS IUPAC Name**	A: [4-[alpha-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride B: bis[[4-[4-(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium] oxalate, dioxalate
Synonyms (state ISO name if available)	Malachite green hydrochloride , Malachite green chloride (A); Malachite green oxalate (B); C. I. Basic green 4; Benzaldehyde green; Acryl brilliant green; Aniline green; China green; Victoria green; Diamond green.
Structural formula of Malachite Green	 (B) (A)

The UK CA proposes that read-across to Malachite Green from Leucomalachite Green is valid. When Leucomalachite Green is present in water, the substance will be ionized. The ionized form of Leucomalachite Green is similar to the structure of Malachite Green. On this basis therefore the aquatic toxicity of Leucomalachite Green will also be similar to Malachite Green. As Malachite Green is not readily biodegradable, Leucomalachite Green will similarly be not readily biodegradable.

10.4 ANNEX IV

UK ENVIRONMENTAL CLASSIFICATION PROPOSAL

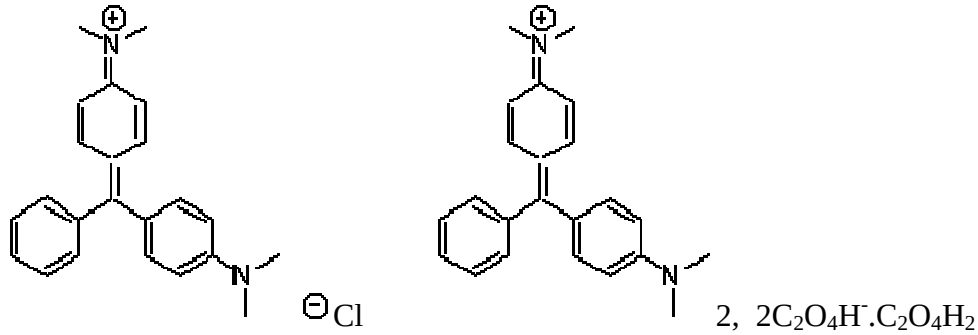
1.1 ECBI/54/02 ADD.8

A: EC No. 209-322-8	**CAS No. 569-64-2**	**ID No.**
B: EC No. 219-441-7	**CAS No. 2437-29-8**	

A: [4-[alpha-[4-(dimethylamino)phenyl]benzylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium chloride

B: bis[[4-[4-(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]dimethylammonium] oxalate, dioxalate

Synonyms
A: Malachite green chloride
B: Malachite green oxalate



(A) (B)

General

- "Malachite green" has three common forms; malachite green (hydro)chloride (A), malachite green oxalate (B) and malachite green base (carbinol).
- Once in water, the chloride and oxalate will dissociate to form the malachite green cation and can, therefore, be treated similarly for the purposes of environmental classification.

Relevant physchem data

- 50 g/l at 80°C (A) (HSE, 2002), 10 g/l at 25°C (B) (DyStar, 2002)
- Log Kow = 0.62 (A) (Hansch et al. 1995)

Relevant ecotoxicity data

- An Environmental Quality Standard annual average = 0.5 µg/l and a maximum allowable concentration (MAC) = 100 µg/l (Burchmore and Wilkinson, 1993) has been set in the UK for malachite green. These have been based on a 96-h LC50 = 0.03 mg/l for *Lepomis macrochirus* (Bills et al., 1977). Other results quoted in the report include (i) 48-h EC50 (*Daphnia magna*) = 0.29 mg/l, (ii) 96-h LC50 (*Pimelas promelas*) = 0.12 mg/l and (iii) 96-h LC50 (*Ictalurus punctatus*) = 0.14 mg/l. Several other studies show L(E)C50s < 1 mg/l. In all studies, the

L(E)C50s are based on nominal concentrations and may have been affected by impurities in the substance. According to the report, results should be treated with this in mind but have been considered valid for the purposes of setting an EQS (Burchmore and Wilkinson, 1993).

- 96 h fish (*O. mykiss*) LC50 = 0.26 mg/l (A) (van Heerden, 1995). No details are available on the test and a judgement on quality cannot be made.
- 96 h fish LC50 = 0.1 – 1 mg/l (B). No details available on the test and a judgement on quality cannot be made (DyStar, 2002).
- Based on weight of evidence, the substance appears to be very toxic to aquatic life with L(E)C50s < 1 mg/l. The information on the available tests is insufficient to allow setting of specific concentration limits.

Relevant fate data

- Biodegradability defined as "< 10%" but no further information given (B) (DyStar, 2002).
- EPIWIN v 3.05 predicts that (A) will not biodegrade fast.
- In natural fresh waters, malachite green cation will combine with available hydroxide to form the colourless, poorly water soluble carbinol form (Alderman, 1985). No information was found on the rate of this reaction and the consequences for classification cannot be determined at this stage.

Environmental Classification Proposal:
N; R50-53, S60, S61

Summary of Proposal

Classification	Toxicity	Degradation	Bioaccumulation	Escape clause
N, R50-53	Data	Default in the absence of data (NRB)	Log Kow < 3	Not applicable

References

- HSE (2002) – human health CPL proposal (ECB1/54/02)
- DyStar (2002) – MSDS (ECB1/54/02 a.7)
- Van Heerden et al. (1995), LC50 determination for malachite green and its effects on certain blood parameters of a catfish, Water SA, 21, p87-94
- Burchmore and Wilkinson (1993), Proposed EQSs for Malachite Green in Water, DoE 3167-2
- Bills et al. (1977), Malachite green: its toxicity to aquatic organisms, persistence, and removal with activated carbon, US Dept. of the Interior Fish and Wildlife Service, Investigations in Fish Control, Part 75
- Alderman (1985), Malachite green: a review. J Fish Diseases, 8, p289-298.

10.5 ANNEX V

This annex shows the final classification decision for leucomalachite green as agreed by the TC C&L in May 2008

EUROPEAN COMMISSION
DIRECTORATE GENERAL - JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Unit: Toxicology and Chemical Substances
European Chemicals Bureau

Follow-up III

Ispra, 29 May 2008

Follow-up III of the meeting of the Technical Committee on Classification and Labelling in Arona,

26-28 September 2007

The comments from FUII have been integrated into the document.
Changes are high-lighted in yellow.
Conclusions and issues completed are high-lighted in turquoise.

1.1 SUBSTANCES FOR WHICH FINAL RECOMMENDATIONS FOR CLASSIFICATION AND LABELLING FOR HEALTH EFFECTS HAS BEEN AGREED

24 substances/group of substances concluded ⇨ Next ATP (1st ATP of the CLP Regulation)
3 substances/groups of substances concluded ⇨ No further action

3 substances/groups of substances for which environmental classification still has to be discussed

L015 **TBHP; Tert-butyl hydroperoxide [(containing > 30% water)] (NL)** **CAS: 75-91-2** **EC No: 200-915-7** **Not in Annex I**	*In October 2006* the substance was discussed for the first time based on the NL proposal. NL had sent in a second revision of their C&L proposal (ECBI/03/06 Rev.2) and reactions to the written comments received during the preparation period in ECBI/03/06 Add. 8. At the meeting in October 2006 the TC C&L agreed that the name of the substance should be “TBHP in 30% water” and that a splitting of entries (suggested by D) was not necessary since the substance was marketed only in this form.

Classification: O; R7 *Agreed 1006* R10 *Agreed 1006* Muta. Cat. 3; R68 *Agreed 0907* T; R23 *Agreed 1006* Xn; R21/22 *Agreed 1006* C; R34 *Agreed 1006* R43 *Agreed 1006* N; R51-53 *Agreed 0406* **Specific concentration limits:** Xi; R37: 5% ≤ C < 10% *Agreed 1006* **R43: C ≥ 0.1%** ***Agreed 1006*** **Labelling:** O, T, N R: 7-10-21/22-23-34-43-68-~~50~~51/53 S: 3/7-14-26-36/37/39-45-~~60~~61 **Classification assigned in accordance with the CLP Regulation:** Org. Perox. EF; H242 Flam. Liq. 3; H226 Muta. 2; H341 Acute Tox. 2; H330 Acute Tox. 3; H311 Acute Tox. 4; H302 Skin Corr. 1C; H314 Skin Sens. 1; H317 Eye dam. 1; H318 Aquatic Chronic 2; H411 **Specific concentration limits:** Skin Sens. 1; H317: C ≥ 0.1% (Xi; R37: 5% ≤ C < 10%]	Although a majority of the TC C&L agreed to apply Muta. Cat. 3; R68 the recommendation is only provisional in order to give MS the time to reflect further on the issue within the follow up period. All other endpoints were agreed as proposed by the NL rapporteur. *Member States not agreeing to Muta. Cat. 3; R68 were asked to react during Follow-up period else the provisionally classification will be regarded as a final classification proposal from the TC C&L.* **BE** did not support classification with R68 because, typically the substance is only a local mutagen and not a systemic mutagen. It is not the first time we have such a case: for example, the pesticide dichlorvos is also a local mutagen and not a systemic mutagen and it is not classified for mutagenicity. Could it be possible to raise the question in the pesticide group (meeting November)? It seems us very important to have the same approach in the two groups when classifying substances to avoid any inconsistency. **NL** responded to the BE comment in document ECBI/03/06 Add. 9 The **DE** position for not to classify with R68 is still the same and was explained in written before. **UK** agreed with BE that it is important to ensure consistency in classification in these cases. TBHP is an in vitro mutagen but has tested negative in standard in vivo tests. The concern, leading to R68, was that because of the reactivity of TBHP these negative findings may be a false negative due to insufficient exposure of the tissues examined (bone marrow) and TBHP might still be a mutagen at the local site of contact (e.g. in the lungs following inhalation or skin) and given that local mutagenicity has not been investigated this is a remaining concern. It may be appropriate to have a discussion on the general issue of how such substances should be classified for mutagenicity. **NL** sent a revised proposal for TBHP, including the GHS classification in document ECBI/03/06 Rev. 3. The rationale for the presented GHS classification can be found there. In addition NL proposes to use STOT 1 or 2 for "Corrosive to the respiratory tract". Furthermore NL confirmed that the GHS classification should be Org. Perox. EF for O; R7. **DK** sent their position in support of classification

Translation of this SCL not necessary as the new GCL for Corrosive substances is 5 %.)	mutagenicity in document ECBI/03/06 Add. 10 distributed with Rev. 2 of the September agenda. In addition they suggest classifying with Carc. Cat. 3; R40. **Conclusion Follow-up:** Based on the comments by BE, DK, DE and UK, mutagenicity should be re-discussed at the September 2007 meeting. *MS were invited to send further comments/positions within the deadlines for the September meeting to facilitate the discussions. In addition MS were asked to react in written prior the meeting in case they supported to further discuss carcinogenicity as suggested by DK.* There was no additional support for further discussion of carcinogenity. NL presented their position on mutagenicity and carcinogenicity together with a summary of new studies in document ECBI/03/06 Add. 11 distributed with Revision 5 of the agenda. They support Muta.Cat.3; R68 (and Muta. 2 H341) and state that the data available is insufficient for classification for carcinogenicity. ***In September 2007*** the TC C&L agreed to confirm the provisional classification for Muta. Cat. 3; R68 (Muta. 2 H341) from the last meeting, and not to classify with STOT 1 or 2 under the CLP Regulation as the effects were already covered by the agreed classification. **NL:** There is a difference in the follow-up document I for the environmental classification between the classification and the labelling. Could you please check this? Does this affect the S-sentences? **ECB**: Yes, thanks this is correct. It should be R51-53 and S61. **⇨ Next ATP**
I025 (N) **4-tert-butylphenol** **Not listed in Annex I** **CAS No: 98-54-4** **EC No: 202-679-0** **Classification:** Repr. Cat. 3; R62 *Agreed 0907* Xi; R37/38 – R41 *Agreed 0306* N ; R51-53 *Agreed 0905* **Labelling:** Xn R: 37/38-41-62-51/53	*March 2006:* Reproductive toxicity **N** had made a classification proposal including classification for both endpoints for reproductive toxicity, Repr. Cat. 3; R62-63 (ECBI/16/06 Add. 1). The discussion was postponed as a 2-generation study had not yet been evaluated by the TC NES. **IND** had provided the TC C&L with a summary of the 2 generation study (ECBI/16/06 Add. 4) distributed with FU III of the March 2006 meeting. *In October 2006* the TC C&L agreed provisionally not to classify the substance as R63 (development) and to classify the substance as R62 (fertility). A lot of questions arose regarding the 2-generation study (Clubb and Jardine, 2006) on which the Norwegian proposal for the application of R62-63 was based and for which a summary had been made available

S: (2-)26-36/37-39-61 **<u>Classification assigned in accordance with the CLP Regulation:</u>** Repr. 2; H361f STOT Single 3; H335 Skin Irrit. 2; H315 Eye Dam. 1; H318 Aquatic Chronic 2; H411	to the TC C&L. The relevant part of the RAR, where the study by Clubb and Jardine, 2006 is described has been submitted by **N** (ECBI/16/06 Add. 5). *MS experts were asked to respond during the written procedure if the provisional agreement of the October 2006 meeting could be confirmed.* **S and NL** agreed to the provisionally agreed classification proposal for reprotoxicity i.e. Repr. Cat. 3; R62. **IND** sent a review on reprotoxicity of 4-tert-butylphenol for consideration at the September meeting in document ECBI/16/06 Add. 6 (MS only), supporting no classification for both fertility and developmental effects. **UK** would like to discuss the reprotoxicity of 4-tert-butylphenol on basis of the review distributed by Industry. **F** support the provisional classification agreed at the October 2006 meeting: - Category 3 for fertility because of the decrease in ovary weight and the atrophy of vaginal epithelium in the high-dose group in the both generations and in the mid-dose group in the first generation. It was accompanied by a slight reduction in implantation sites in the high-dose groups that is not within the historical control incidence in the F1 females. Besides, the decrease of ovary weight in the high-dose F1 females was more severe (-28%) than the general decrease of body weight (-17% during pre-mating and -13% during the lactation period) and it can not be attributed to a secondary effect. - No classification for development because the effect seen on pups survival at the first generation were not reproduced at the second generation. **BE:** After examination of the documents received from N and a detailed analysis of the effects, BE would like to have a verbal discussion concerning this substance at the next meeting for the fertility classification. On basis of the new document by IND and the response from UK and BE, it was decided to discuss reprotoxicity of 4-tert-butylphenol at the September 2007 meeting. *MS were invited to send further comments/positions within the deadlines for the September meeting to facilitate the discussions.*

	No further comments received. ***In September 2007*** the TC C&L agreed to confirm the provisional classification for Repr. Cat. 3; R62 (Repr. 2 H361f) from the last meeting, and they also confirmed that it would not be necessary to classify for developmental effects. ⇨ **Next ATP**
W034 **N-Cyclohexylbenzothiazol-2-sulphenamide (DE)** **613-136-00-6** **EC: 202-411-2** **CAS: 95-33-0** **Current Classification and proposal:** NC Repr. Cat. 3; R62 *Agreed 0907* R43 *Agreed 1006* N; R50-53 *Agreed 0905* **Labelling:** Xi, N R: 43-50/53 S**:** (2-)24-37-60-61 **Classification assigned in accordance with the CLP Regulation:** Skin Sens. 1; H317 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	***October 2006:*** In document ECBI/44/06 DE proposed to keep the current R43 classification. **FR** has sent in the following comment (ECBI/87/06 Add. 1): Skin sensitisation: positive reactions in humans were observed in several patch test studies using a CBS concentration of 1%, the default cut-off for R43 classification of preparation and we would like to discuss the relevance of a lower specific concentration limit. At the *October 2006* meeting the TC C&L agreed to keep the current classification and not to add SCLs for R43. **NL** reported that there was a discussion about fertility at the TC NES. Therefore they would send in a proposal for the application of R62 in the Follow-up period. **IND** sent in a late document ECBI/44/06 Add. 1 where they object against application of SCLs for R43 (at the meeting it was already agreed not to apply SCLs for R43). **NL** provided their classification proposal for R62 (ECBI/44/06 Add. 2), which was supported by BE, DK and S. **BE:** this classification is based on the assumption of hydrolysis of CBS to equimolar amounts of CHA and MBT, in the gastro-intestinal tract, based on data in rat. We can consider that this substance may cause concern for fertility and could accept the classification proposed by NL if this classification is proposed with SCL. There are sufficient data on CHA and on CBS, like explained in the RAR report, to propose SCL of 25 %. **DK** support the NL proposal for R62, due to the fact that the fertility of rat is very high. In order to see any effects on fertility of rat relative high doses are needed. Impact on human fertility may be more sensitive than on rat. **F:** The effects of cyclohexylamine (CHA) on fertility were discussed in the TC C&L of March 2006 and a classification cat. 3; R62 was agreed on

	the basis of the studies discussed in the NL proposal for classification of CBS for fertility (document ECBI/44/06 Add. 2). The main question is therefore the relevance of CHA data for the effects of CBS and additional data on the rate of hydrolysis of CBS into CHA would be useful to provide a final position. **S:** We support the classification Repr. Cat. 3; R62 proposed by NL. There is evidence from the literature that testicular atrophy and also reduced fertility occur in the rat after administration of cyclohexylamine, a metabolite to N-cyclohexylbenzothiazol-2-sulphenamide. See ECBI/44/06 Add. 3 Based on the support from BE, S and DK for the NL proposal, R62 and specific concentration limits for R62 should be discussed at the September 2007 meeting. **IND** sent arguments to explain that reprotoxicity classification is not warranted for CBS with document ECBI/44/06 Add. 4. *DE was asked to provide data on the hydrolysis of CBS into CHA, on request by F.* IND provided the requested information on the hydrolysis study in documents ECBI/44/06 Add. 5 which was distributed with Rev. 5 of the agenda. *MS were invited to send further comments/positions within the deadlines for the September meeting to facilitate the discussions.* No further comments/positions were received. ***In September 2007*** the TC C&L agreed not to classify the substance for fertility effects based on the available data including the new hydrolysis data and therefore the discussions on specific concentration limits for this endpoint also become irrelevant. **⇨ Final classification proposal, no action needed**
M012 **2-ethoxy-2-methylpropane (ETBE) (FIN)** **EC: 211-309-7** **CAS: 637-92-3** **Classification:** F; R11 *Agreed 1006*	*In October 2006* the TC C&L agreed to classify the substance as R11 and not to classify for narcotic effects (R67) as suggested in the FIN proposal, however, during the follow up procedure several MS experts indicated support for R67 classification. **DK:** We still support the original classification proposal from FIN to assign R67 and we would like to re-open the discussion.

R67 *Agreed 0907* **<u>Labelling proposal:</u>** F R: 11-67 S: (2-)9-16-24 **<u>Classification assigned in accordance with the CLP Regulation:</u>** Flam. Liq. 2; H225 STOT Single 3; H336 (GHS classification confirmed by FIN)	**FIN:** We still feel that there is a case for R67. In the 28-d study with rats CNS-effects were seen already after 3 h which is below the 4 h condition in the criteria: Signs of general sedation and reduced motor activity were noted in rats exposed to 4000 ppm ETBE vapour, with some animals exhibiting mild to moderate ataxia. After 3 hours exposure no startle response was evident in the majority of high exposure animals. All treated animals appeared to be in 'sleeping position' (muscle relaxation not evident) during exposure but were normal 15 minutes post-exposure. This transient clinical observation could well be interpreted as a narcotic effect. The effect-concentration of 4000 ppm (17 mg/L air), correspond to a ratio of the effect concentration at < 4 h to the saturated vapour concentration (ETBE-SVC: 163 000 ppm, 20 C) of < 1/10 (ETBE-ratio: 0,02). Therefore, the criteria are fully met. Additionally in support of the mentioned findings, in the 90-d study with mice, transient ataxia was occasionally observed post-exposure at 5000 ppm animals for both sexes. In the 90-day inhalation study in rats, a transient ataxia was noted in high dose males only, post-exposure only. In the neurotoxicology substudy (Dorman et al., 1997) it is stated: "Transient ataxia, a sign of narcosis, was notes in male rats immediately following the 6-h exposure to 5000 ppm ETBE. Statistically significant treatment effects on motor activity were not observed. Minor changes in grip strength and hindlimb splay were observed; however, none demonstrated a dose-response relationship or a consistent pattern of neurological dysfunction. .... Although ataxia was a common feature of acute ETBE neurotoxicity in rats following high-level exposure, adverse neurological effects are not expected in the general public at the anticipated exposure levels associated with automotive refueling." Therefore, the overall weight of evidence is pointing towards a narcotic effect which justifies the additional R-phrase 67. **BE :** agrees with R67, the second criteria is effectively met (SVC between 109000 and 185000 ppm, depending on the BP considered) **NL** agrees with R67 as well. **DK** sent, in January 2007, with ECBI/83/06 Add. 1, a report on read across for Volatile Aliphatic Ethers, and argues for R67 classification for ETBE. R67 was to be re-discussed at the September 2007 meeting due to the support by DK, FIN, BE and NL during the follow-up period. *MS were invited to send further comments/positions within the deadlines for the September meeting to facilitate the discussions.*

	No further comments/positions were received. ***In September 2007*** the TC C&L agreed to assign R67 (STOT Single 3 H336) to the substance based on the original proposal from FIN without any further discussion. **⇨ Next ATP**
U080 **Leucomalachite green** **Not listed in Annex I** **CAS No: 129-73-7** **EC No: 204-961-9** **Classification** Carc. Cat. 3; R40 *Agreed 1006* Muta. Cat 3; R68 *Agreed 0907* Xn, R22 *Agreed 1006* N; R50-53 *Agreed 0905* **Labelling** Xn, N R: 22-40-68-50/53 S: (2-)36/37-46-60-61 **Classification assigned in accordance with the CLP Regulation:** Carc. 2; H351 Muta. 2; H341 Acute Tox. 4; H302* Aquatic Acute 1; H400 Aquatic Chronic 1; H410 * Necessary to check the data for confirmation of the classification.	*In March 2006* the TC C&L agreed that they would like to receive from the UK a more robust back-up for reading across for other end-points (besides carcinogenicity and mutagenicity) from malachite green (Index No: 602-096-00-5) to this substance. UK was requested to send in this information (robust back-up for reading across) during the Follow-up period. During the Follow-up period of the March meeting it was agreed also to re-open the discussion of carcinogenicity of malachite green in context of the reading across in between the two substances. *In October 2006* the TC C&L came to agreement on the classification of leucomalachite green besides for the mutagenicity end-point. D agreed that leucomalachite green should not be classified for mutagenicity. UK wanted to re-examine mutagenicity data during the FU procedure. **UK** gave their position on mutagenicity after the Follow-up period in ECBI/35/05 Add. 1 Rev. 1. Therefore the Mutagenicity discussion is going to be carried forward to the September 2007 meeting. DK: S46 is irrelevant. Applies only to consumer products. Delete systematically. ECB: Does UK confirm that S46 is irrelevant? UK is asked to check the new classification proposal indicated with * to provide a final classification proposal for these end-points. **S** sent comments on reprotoxicity of leucomalachite green in document ECBI/35/05 Add. 2. Although S requests re-opening of reprotoxicity as well, ECB is of the opinion that that discussion is finalised for leucomalachite green. *MS were invited to send further comments/positions within the*

	deadlines for the September meeting to facilitate the discussions. No further comments/positions were received. ***In September 2007*** the TC C&L agreed to classify leucomalachite green with Muta. Cat. 3; R68 (Muta. 2 H341). There was no support of further discussion of the reproductive toxicity classification. During FUI to Sept 2007, the UK commented: DK consider S46 to be unnecessary as it only applies to consumer products and suggest that it is deleted. The same point was made for malachite green at the October 2006 meeting. In the follow-up II ECB concluded that S46 would not be deleted because it could not be confirmed for certain that consumers would not exposed to the substance. We think the same applies to leucomalachite green so we do not support removal of S46. **ECB** concludes that S46 should be applied. **UK is asked to please check if the classification according to the GHS criteria in the CLP Regulation for Acute tox. 4 H302 is justified according to data.** **⇨ Next ATP**
U066 **Malachite green (UK)** **Malachite green hydrochloride;** **Malachite green chloride; [4-[alpha-[4-(dimethylamino) phenyl] benzyl idene] cyclohexa-2,5-dien-1-ylidene] dimethyl ammonium chloride [1]** **Malachite green oxalate; bis[[4-[4-(dimethylamino) benzhydryl idene] cyclohexa-2,5-dien-1-ylidene] dimethyl ammonium] oxalate, di oxalate [2]** **Index No: 602-096-00-5 [1]** **Not listed in Annex I [2]** **CAS No: 569-64-2 [1]** **CAS No: 2437-29-8 [2]** **EC No: 209-322-8 [1]** **EC No: 219-441-7 [2]** **Classification** Repr. Cat. 3; R63 Xn; R22	*In March 2006* the TC C&L did not agree to add Muta. Cat. 3; R68 to the entry. Due to the comments in the FU period on Leukomalachite green, Carcinogenicity of Malachite green was re-discussed together with leucomalachite green at the **October 2006** meeting. **12 *IN PREPARATION FOR THE OCTOBER 2006* TC C&L MEETING UK SUBMITTED THE REQUESTED DOCUMENT ECBI/35/05 ADD. 1 ON THE USE OF READ-ACROSS ARGUMENTS TO FILL DATA GAPS.** *In October 2006* a majority of the TC C&L agreed that classification for mutagenicity would not be necessary based on negative data for malachite green. The TC C&L also agreed that it was not relevant to read across the positive findings in leucomalachite green studies for classification of malachite green. The conclusion of the meeting was then not to change the current classification as listed in Annex I. **UK** reacted only in the preparation period for the March 2007 meeting (which was postponed to September 2007), why a

Xi; 41 N; R50-53 **Labelling:** Xn, N R: 22-41-63-50/53 S: (2-)26-36/37-39-46-60-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 2; H361d Acute Tox. 4; H302* Eye Dam. 1; H318 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 * Necessary to check the data for confirmation of the classification.	final consideration of the mutagenicity/carcinogencity classification for leucomalachite green and malachite green must take place at that meeting. *In preparation for the September 2007* meeting **SE** sent documents ECBI/35/05 Adds. 3, 4, 5, 6 and 7, requesting the re-opening of discussion of carcinogenicity and mutagenicity of Malachite Green, in analogy with Leucomalachite green. *MS experts were asked to indicate their support or non-support for the re-opening of the carcinogenicity and mutagenicity discussion of malachite green in analogy with leucomalachite green. In case there was no support, this substance would be removed from the final agenda.* **NL:** We do not support re-opening of the discussion on the mutagenicity and carcinogenicity of malachite green as there are no new data or argumentations. **FR:** Malachite green and leucomalachite green have very close structures and data on repeated-dose toxicity and carcinogenicity indicate similar target organs and mode of action. Considering their comparable tumour profile FR support the re-opening of the malachite green discussion particularly for carcinogenicity in analogy with leucomalachite green. ***In September 2007*** the TC C&L agreed not to classify malachite green neither for mutagenicty nor carcinogenicity, but they supported the classification already listed in Annex I for the substance. ⇨ **No further action.**
Y009 **Octamethylcyclotetrasiloxane** **Index No: 014-018-00-1** **CAS No: 556-67-2** **EC No: 209-136-7** **Classification:** NC for carcinogenicity *Agreed 0305* Repr. Cat. 3; R62 *Agreed 0907* R53 *ATP 28* *Currently classified in Annex I (ATP 28): Repr. Cat. 3; R62 -*	**Reproductive toxicity (Fertility)** *In November 2005* several Member States wanted to consult Specialised Experts on fertility effects of OMCTS. *In March 2006* the TC C&L agreed on the questions as drafted by S to be forwarded to the Specialised Experts (ECBI/63/05 Rev. 1). *In September 2006* the Specialised Experts discussed the question forwarded by the TC C&L. Their conclusions (ECBI/121/06) was circulated to the group prior the October meeting.

R53 **Labelling**: Xn R: 62-53 S: (2 -)36/37-46-51-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 2; H361f Aquatic Chronic 4; H413	*Conclusions for Octamethylcyclotetrasiloxane (from ECBI/121/06):* *"Inhalation exposure of female rats to D4 around the time of mating causes a dose related reduction of numbers of corpora lutea, implantation sites and litter sizes. These effects occur in the absence of marked maternal toxicity. Inhibition of the LH surge and subsequent ovulation is the mode of action, which is relevant to human. However, the mechanism leading to the inhibition of the LH surge is unknown. There was no experimental data in the rat to support the hypothesised mechanism of hypothalamic norepinephrine inhibition with D4. Therefore it cannot be excluded that the reproductive effects in the rat are relevant to humans.* *A number of Specialised Experts considered that Repr. Cat. 2; R60 was warranted on the basis of the specific effects of D4 on the LH surge in rats which occurred independent of maternal toxicity. In addition, in the absence of knowledge on the mechanism underlying the effect on the LH surge, the effects should be regarded as relevant to humans.* *A similar number of Specialised Experts preferred Repr. Cat. 3; R62. This was because of major differences in the regulation of ovulation in the human as compared to the rat making the relevance to humans doubtful.* *The Specialised Experts assessment of the data did not take account of human exposure and issues related to normal handling and use".* *In October 2006* the TC C&L agreed to postpone the discussion due to the split opinions of the Specialised Experts and await the detailed summary record from the Specialised Experts meeting prior coming to a final classification recommendation. **UK:** The reproductive toxicity of D4 and its relevance to humans was considered recently by the Specialised Experts. We note that it has also been considered by another Commission expert group, the Scientific Committee on Consumer Products (SCCP), in 2005 (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_035.pdf), who concluded that the effects of D4 on fertility in rodents were of little relevance to humans. We do not think that the Specialised Experts were aware of this assessment when they considered D4. Obviously it would be appropriate for the Specisalised Experts and C&L group to consider this expert opinion when deciding on the appropriate classification for D4. **IND** sent documents ECBI/63/05 Add. 3 and 4 on the reprotoxicity findings for OMCTS.

	The detailed Summary Record from the Specialised Experts meeting (ECBI/51/07) was distributed with Revision 3 of the September agenda. **IND** sent their comments to the SE Summary Record and a proposal on further testing in document ECBI/63/05 Add. 5 distributed with Revision 5 of the September agenda. ***In September 2007*** the TC C&L agreed to remain with the current classification of D4 as Repr. Cat. 3; R62. However IND suggested anyway to perform further testing to clarify the mechanism that had caused concern among many TC C&L experts since the first agreement on the classification with Repr. Cat. 3; R62 was reached. The results from the testing would be made available in 6-9 months. ECB agreed to provide the TC C&L with this information when forwarded to them from IND, but of course no further discussion would be possible under the responsibilities of the ECB. UK, SE and NL agreed to look into the data when available and if there would be a concern for re-classification they agreed that one of them would provide ECHA with an Annex XV proposal to re-start the discussion at ECHA. ⇨ **No further action.**
Methyltin compounds: **F049 [1]** **Methyltin trichloride, MMTC** **CAS: 993-16-8** **EC: 213-608-8** **<u>Classification:</u>** Muta. Cat. 3; R68 *Agreed 1006* Repr. Cat. 3; R63 *Agreed 0907* Xn; R22 *Agreed 1006* [N; R50/53] *To be discussed* **<u>Labelling:</u>** Xn R: 22-63-68[-50/53] S: (2-)36/37[-60-61]	*In October 2006* the TC C&L on the basis of the F proposal (ECBI/27/06) it was agreed to classify MMTC for mutagenicity in category 3 and with Xn; R22 for acute toxicity. It was agreed not to classify for corrosivity and repeated dose toxicity. *In October 2006* the TC C&L on the basis of the F proposal (ECBI/26/06 Rev. 1) it was agreed to classify MMT(EHMA) for mutagenicity in category 3 and with Xn; R22 for acute toxicity. It was agreed not to classify for sensitisation and repeated dose toxicity. (*In October 2006* the discussion of the classification for the two dimethyltin compounds: Dimethyltin dichloride, DMTC (EC No: 212-039-2, CAS No: 753-73-1) and Dimethyltin bis(2-ethylhexyl- mercaptoacetate, DMT(EHMA) (EC No: 260-829-0, CAS No: 57583-35-4) were concluded) **IND** gives in their paper ECBI/27/06 Add. 1 information on maternal toxicity and reprotoxicity of MMTC. Document ECBI/27/06 Add. 2 is a scientific paper on Evaluation of developmental neurotoxicity of organotins via drinking water in rats. Furthermore the following documents were sent by

Classification assigned in accordance with the CLP Regulation: Muta. 2; H341 Repr. 2; H361d Acute Tox. 4; H302 [Aquatic Acute 1; H400] [Aquatic Chronic 1; H410] FR confirms that the acute tox. data are consistent with the classification shown. **F051 [2]** **Methyltin tris(2-ethylhexyl-mercaptoacetate, MMT(EHMA)** **CAS: 57583-34-3** **EC: 260-828-5** **Classification:** Muta. Cat. 3; R68 *Agreed 1006* Repr. Cat. 3; R63 *Agreed 0907* Xn; R21/22 *Agreed 0907/1006* [NC for ENV] *To be discussed* **Labelling:** Xn R: [21]/22-63-68[-50/53] S: (2-)36/37[-60-61] **Classification assigned in accordance with the CLP Regulation:** Muta. 2; H341 Repr. 2; H361d Acute Tox. 4; H312 Acute Tox. 4; H302 *ENV still to be discussed* FR confirms that the acute tox. data are consistent with the classification shown.	IND: ECBI/27/06 Add. 3 parts I, II, III and IV on reprotoxicity of MMTC as well. **S** commented by email on the reprotoxicity of MMTC (ECBI/27/06 Add. 4) and re-submitted the expert report ECBI/30/04 and the Guidelines for Developmental Toxicity Risk Assessment from the EPA (ECBI/27/06 Add. 5). **IND** sent further information requested by the TC C&L in documents ECBI/27/06 Add. 6 (I-IV) and ECBI/27/06 Add. 7 (I, II) distributed with Revision 2 of the September agenda *MS were asked to send their comments to the new information forwarded by IND within the deadlines for the September meeting.* **F** sent further comments developmental toxicity in their document ECBI/27/06 Add. 8 confirming their position to classify both substances with Repr. Cat. 3; R63. ***In September 2007*** the TC C&L agreed to classify MMTC and MMT(EHMA) with Repr. Cat. 3; R63 (Repr. 2 H361d). In addition it was agreed to classify MMT(EHMA) with Xn; R21. ⇨ **Next ATP if ENV classification is concluded.** ECB will evaluate whether to make a written procedure and ask the TC C&L Environmental experts to agree on classification for F049 (N; R50-53 proposed by FR in ECBI/27/06) and F051 (NC proposed by FR in ECBI/26/06) for environment, else the partial classification concerning the environment should be handed over for discussion at ECHA with support of an Annex XV dossier. **After FU II:** **A written procedure for ENV has not been made and consequently the issue of classification of these substances for environmental effects will be discussed further.** ⇨ **Hand-over to ECHA**

D147 **Imidazole** **EC: 206-019-2** **CAS: 288-32-4** **Classification:** Repr. Cat. 2; R61 *Agreed 0907* Xn; R22 *Agreed 1006* C; R34 *Agreed 1006* **Labelling:** T R: 61-22-34 S: 53-45 **Classification assigned in accordance with the CLP Regulation:** Repr. 1B; H360D Acute Tox. 4; H302 Skin Corr. 1B; H314	*In October 2006* the TC C&L agreed to classification for acute toxicity and corrosivity on the basis of the DE classification proposal (ECBI/59/06). **Reproductive toxicity (developmental effects)** MS experts requested more time to evaluate the data on reprotoxicity. The discussion of this end-point will therefore continue at the September 2007 meeting. **DE** provided a revised C&L proposal for Imidazole, ECBI/59/06 Rev. 2, providing more information on the frequency of occurrence of cleft palate in the reported studies. For the third revision of the agenda **DE** provided the C&L proposal in Annex XV format (ECBI/59/06 Rev. 3). The GHS classification was confirmed by DE in their C&L proposal. *DE was also requested to provide a summary of the flammability tests to the TC C&L, to be added to the proposal for completeness.* *MS were invited to send further comments/positions within the deadlines for the September meeting to facilitate the discussions.* No further information/comments/positions were received. ***In September 2007*** the TC C&L agreed to the classification for Repr. Cat. 2; R61 (Repr. 1B H360D) based on the DE proposal. ⇨ **Next ATP**
Cadmium diformate; **Cadmiumformate** **Index No: 048-003-00-6** **CAS No: 4464-23-7** **EC No: 224-729-0** **Classification:** Carc. Cat. 2; R45 *Agreed 0907* T; R23/25 *ATP29* ~~R33~~ (covered by note H) N; R50-53 *ATP29* **Note H**	Preparing the corrigendum of Annex I it was reported that the classification of 4 cadmium compound entries in Annex I classified with Xn; R68 should be changed into Carc. Cat. 3; R40. In *March 2006* TC C&L confirmed that this had been the intention but that the classifications should be re-discussed based on the classification in category 2 for carcinogenicity made for some other cadmium compounds listed in the draft 30th ATP list. *Follow-up of March 2006:* S: **At the March meeting a corrigendum of Annex I for four cadmium compounds was proposed. Some Cd compounds have already been updated with labelling for**

Currently classified in Annex I (ATP 29): T; R23/25-R33-Xn; R68-N; R50-53 *Specific Concentration Limits (ATP 29):* *C ≥ 25 %: T, N; R23/25-33-50/53-68* *10 % ≤ C < 25 %: T, N; R23/25-33-51/53-68* *2,5 % ≤ C < 10 %: Xn, N; R20/22-33-51/53-68* *1 % ≤ C < 2,5 %: Xn; R20/22-33-52/53-68* *0,1 % ≤ C < 1 %: Xn; R20/22-33-52/53* *0,25 % ≤ C < 0,1 %: Xn; R20/22-33-52/53* *(error in the general limits for environmental classification ~~– to be corrected as revised)~~* ~~Specific Concentration Limits:~~ ~~C ≥ 25 %: T, N; R45-23/25 [33 ?] 50/53~~ ~~10 % ≤ C < 25 %: T, N; R45-23/25 [33 ?] 51/53~~ ~~2,5 % ≤ C < 10 %: T, N; R45-20/22 [33 ?] 51/53~~ ~~0,25 % ≤ C < 1 %: T; R45-20/22 [33 ?] 52/53~~ ~~0,1 % ≤ C < 0,25 %: T; R45-20/22 [33 ?]~~ ~~[0,01 % ≤ C < 0,1 %: T; R45 ?]~~ **Labelling:** T; N R: 45-23/25-~~33~~ 50/53 S: 53-45-60-61 **Specific concentration limit (presented in accordance with the CLP Regulation): Carc. Cat. 2; R45: C ≥ 0,01 %** **Classification assigned in accordance with the CLP Regulation:** Carc. 1B; H350 Acute Tox. 3; H331* Acute Tox. 3; H301* Aquatic Acute 1; H400 Aquatic Chronic 1; H410 **Note H** *Necessary to check the data for confirmation of the classification. **Specific Concentration Limit:** ~~ECB proposes:~~ ~~[C ≥ 10 %: Acute Tox 3* H301, H331~~ ~~0,1 % ≤ C <10%: [STOT Rep. 2, H373 ?] ;~~	**cancer which was also proposed at the meeting but the classification for reprotoxic effects has not been addressed. A read-across (if studies are not available) should also be made to reproductive toxicity in line with the already updated entries in Annex I with Repr. Cat. 2; R60/61 or Repr. Cat. 3; R62/63.** DK **agrees upon read-across regarding reproductive toxicity.** In *October 2006* it was agreed by the TC C&L that read across in principle would be possible, but that it should be examined more carefully for which endpoints. **DE** provided a table with solubility data for cadmium compounds (ECBI/61/06 Add. 2). *The TC C&L was asked to provide their opinions in written within the deadlines of the September meeting on the issue for which of the endpoints read across could be applied.* No further comments/positions were received. ***In September 2007*** the TC C&L agreed to revise this entry and classify it with Carc. Cat. 2; R45 (Carc 1B H350) based on the solubility data, which indicated that the compound was easily soluble. The toxicity would then be due to the presence of Cadmium ions and it was correct to classify this substance as other easily soluble Cadmium compounds already listed in Annex I. It was further agreed not to read across any other end-points such as for reproductive toxicity at this point in time, but in case a Member State had such a concern they should provide ECHA with an Annex XV dossier for re-discussion in the future. **After FUI:** **DE**: As only R45 has been crossread should not the entry be assigned Note H? **NL**: It was decided to classify for R45 based on read-across but not to read-across for other endpoints for practical reasons. As classification for other endpoints based on read-across cannot be excluded it is proposed to add note H. **ECB** will ask MS to react if they do NOT agree to add Note H. DE**: To agree on SCL according to CLaP may be very**

~~Acute Tox 4* H302, H332~~ **Carc 1B H350:C ≥ 0,01 %**	**difficult in a written procedure. As only R45 was added, it could be discussed that the 0.01% SCL for the highly soluble Cd compounds should be crossread also.** ECB **will ask MS if the SCL of 0.01 % for R45 will be read across from the other highly soluble Cd compounds in Annex I.** **For SCL setting under CLaP, there may be no easy consensus for acute toxicity SCL [I would delete them as the CLaP formula in 3.1.3.6.1 automatically sets SCL because the LD/LC_{50} is included in the calculation; if not human data indicate necessity to deviate].** ECB **suggests to "translate" the SCLs also for acute toxicity to Annex VI for the time being in order not to loose this important hazard information, meanwhile the "issue" with the ATE formula in the GHS is being considered (the GHS formula does not take into account a SCL as currently in the EU).** **STOT SCL (R33) could be crossread as there are also SCL re-agreed on R48 for Cd sulphate and Cd chloride in the 29.ATP. ECB: 0.1% is already ascribed the entry for R33 and R20/22.** ECB: **ECB has looked at the other entries in Annex I of soluble Cd compounds (e.g. the Cd chloride, Cd fluoride, Cd sulphate) which all are classified with T; R48/23/25 (that will be translated into STOT Rep 1 H372). However, if R33 will be kept for this easily soluble compound (see above) an inconsistency with regard to the other soluble Cd compounds (will remain in Annex I and) will be introduced in Annex VI to the GHS CLP Regulation, since R33 is suggested to be translated into STOT Rep. 2. ECB doubts that this inconsistency is justified in view of the well-known, documented and typical effects of Cd on e.g. the kidney and bone changes/damages also demonstrated in humans, caused by the Cd-ion after repeated exposure. These effects observed in humans (and animals), would rather justify STOT Rep. 1.** **Of the reasons above, ECB suggests either to delete R33 and include Note H or to change R33 to R48/23/25, which should translate to STOT Rep. 1 H372.** ECB: **In summary, MS are asked during the FU to react:** **- if you do NOT agree to Note H to be assigned the entry in**

	accordance with the above proposal from DE and NL to allow consideration of classification also for other endpoints **-if the SCL should be cross read from the highly soluble Cd compounds, i.e. 0.01% for R45** **- if also the SCLs for acute toxicity should be translated to the CLP classification (as proposed in left column)** **- if R33 should be deleted and covered by an addition of Note H** **- if R33 should be changed to T; R48/23/25, which should translate to STOT Rep. 1** **After FUII:** **BE: - how does Note H translate in CLR?** - agree [on SCL 0,01% for R45] for cadmium diformate as this chemical is soluble - SCLs for acute toxicity [are] useless as the specific concentration limits are intended for mixtures and in CLR, the later will be classified according to the additivity formula for acute toxicity - agrees with the ECB reasoning [for R33]. If the chemical would have been classified in CLR, it would have been most probably assigned STOT Rep 1. **DE: R33 crossreading including SCL; there was no consensus on cross-reading on other hazard classes than carcinogenicity at the meeting. So … to be consistent would not to translate the R33 into STOT Rep. 1 but to add Note H for all Cd compounds under discussion.** ECB Conclusion: There are no objections to addition of Note H. There is no strong support for changing R33 into T; R48/23/25. As Note H is already considered appropriate, it can also cover R33 (STOT). There is support for the SCL for carcinogenicity and no objections, a SCL 0,01% is therefore added. **⇨ Next ATP**
Cadmium cyanide **Index No: 048-004-00-1** **CAS No: 542-83-6** **EC No: 208-829-1**	Preparing the corrigendum of Annex I it was reported that the classification of 4 cadmium compound entries in Annex I classified with Xn; R68 should be changed into Carc. Cat. 3; R40.

Classification: Carc. Cat. 2; R45 *Agreed 0907* T+; R26/27/28 *ATP29* R32 *ATP29* ~~R33~~ (covered by note H) N; R50-53 *ATP29* **Note H** *Currently classified in Annex I (ATP 29): T+; R26/27/28 - R32 - R33 - Xn; R68 - N; R50-53* *Specific Concentration Limits(ATP 29):* *C ≥ 25 %: T+, N; R26/27/28-32-33-50/53-68* *7 % ≤ C < 25 %: T+, N; R26/27/28-32-33-51/53-68* *2,5 % ≤ C < 7 %: T, N; R23/24/25-32-33-51/53-68* *1 % ≤ C < 2,5 %: T; R23/24/25-32-33-52/53-68* *0,25 % ≤ C < 1 %: Xn; R20/21/22-33-52/53* *0,1 % ≤ C < 0,25 %: Xn; R20/21/22-33* ~~Specific Concentration Limits:~~ ~~C ≥ 25 %: T+, N; R45-26/27/28-32-33-50/53~~ ~~7 % ≤ C < 25 %: T+, N; R45-26/27/28-32-33-51/53~~ ~~2,5 % ≤ C < 7 %: T, N; R45-23/24/25-32-33-51/53~~ ~~1 % ≤ C < 2,5 %: T; R45-23/24/25-32-33-52/53-68~~ ~~0,25 % ≤ C < 1 %: T; R45-20/21/22-33-52/53~~ ~~0,1 % ≤ C < 0,25 %: T; R45-20/21/22-33~~ **Labelling:** T+; N R: 45-26/27/28-32-33-50/53 S: 53-45-60-61 **Specific concentration limit (presented in accordance with the CLP Regulation): Carc. Cat. 2; R45: C ≥ 0,01 %** **Classification assigned in accordance with the CLP Regulation:** Carc. 1B; H350	In *March 2006* TC C&L confirmed that this had been the intention but that the classifications should be re-discussed based on the classification in category 2 for carcinogenicity made for some other cadmium compounds listed in the draft 30th ATP list. *Follow-up of March 2006:* S: **At the March meeting a corrigendum of Annex I for four cadmium compounds was proposed. Some Cd compounds have already been updated with labelling for cancer which was also proposed at the meeting but the classification for reprotoxic effects has not been addressed. A read-across (if studies are not available) should also be made to reproductive toxicity in line with the already updated entries in Annex I with Repr. Cat. 2; R60/61 or Repr. Cat. 3; R62/63.** DK **agrees upon read-across regarding reproductive toxicity.** In *October 2006* it was agreed by the TC C&L that read across in principle would be possible, but that it should be examined more carefully for which endpoints. **DE** provided a table with solubility data for cadmium compounds (ECBI/61/06 Add. 2). *The TC C&L was asked to provide their opinions in written within the deadlines of the September meeting on the issue for which of the endpoints read across could be applied.* No further comments/positions were received. ***In September 2007*** the TC C&L agreed to revise this entry and classify it with Carc. Cat. 2; R45 (Carc 1B H350) based on the solubility data, which indicated that the compound was easily soluble. The toxicity would then be due to the presence of Cadmium ions and it was correct to classify this substance as other easily soluble Cadmium compounds already listed in Annex I. It was further agreed not to read across any other end-points such as for reproductive toxicity at this point in time, but in case a Member State had such a concern they should provide ECHA with an Annex XV dossier for re-discussion in the future. **After FUI:** **DE**: As only R45 has been crossread should not the entry be

Acute Tox. 2; H330* Acute Tox. 1; H310 Acute Tox. 2; H300* Aquatic Acute 1; H400 Aquatic Chronic 1; H410 EUH032 *Necessary to check the data for confirmation of the classification. ~~[Specific Concentration Limits?]~~ ~~**See ECB proposal below:**~~ **Specific Concentration Limit:** ~~**ECB proposes:**~~ ~~**[C ≥ 10 %: Acute Tox 3* H301, H331**~~ ~~**0,1 % ≤ C <10%: [STOT Rep. 2, H373 ?] ;**~~ ~~**Acute Tox 4* H302, H332**~~ ~~**0,01 % ≤ C <0.1%: Carc 1B H350]**~~ **Carc 1B H350:C ≥ 0,01 %**	assigned Note H? **NL**: It was decided to classify for R45 based on read-across but not to read-across for other endpoints for practical reasons. As classification for other endpoints based on read-across cannot be excluded it is proposed to add note H. **ECB** will ask MS to react if they do NOT agree to add Note H. DE**: To agree on SCL according to CLaP may be very difficult in a written procedure. As only R45 was added, it could be discussed that the 0.01% SCL for the highly soluble Cd compounds should be crossread also.** ECB **will ask MS if the SCL of 0.01 % for R45 will be read across from the other highly soluble Cd compounds in Annex I.** **For SCL setting under CLaP, there may be no easy consensus for acute toxicity SCL [I would delete them as the CLaP formula in 3.1.3.6.1 automatically sets SCL because the LD/LC$_{50}$ is included in the calculation; if not human data indicate necessity to deviate].** ECB **suggests to "translate" the SCLs also for acute toxicity to Annex VI for the time being in order not to loose this important hazard information, meanwhile the "issue" with the ATE formula in the GHS is being considered (the GHS formula does not take into account a SCL as currently in the EU).** **STOT SCL (R33) could be crossread as there are also SCL re-agreed on R48 for Cd sulphate and Cd chloride in the 29.ATP (ECB: 0.1% is already ascribed the entry for R33 and R20/22) .** ECB**: ECB has looked at the other entries in Annex I of soluble Cd compounds (e.g. the Cd chloride, Cd fluoride, Cd sulphate) which all are classified with T; R48/23/25 (that will be translated into STOT Rep 1 H372). However, if R33 will be kept for this easily soluble compound (see above) an inconsistency with regard to the other soluble Cd compounds (will remain in Annex I and) will be introduced in Annex VI to the GHS CLP Regulation, since R33 is suggested to be translated into STOT Rep. 2. ECB doubts that this inconsistency is justified in view of the well-known, documented and typical effects of Cd on e.g. the kidney and bone changes/damages also demonstrated in humans, caused by the Cd-ion after repeated exposure. These effects observed in humans (and animals), would rather justify STOT Rep. 1.** **Of the reasons above, ECB suggests either to delete R33**

	and include Note H or to change R33 to R48/23/25, which should translate to STOT Rep. 1 H372. ECB: **In summary, MS are asked during the FU to react:** **- if you do NOT agree to Note H to be assigned the entry in accordance with the above proposal from DE and NL to allow consideration of classification also for other endpoints** **-if the SCL should be cross read from the highly soluble Cd compounds, i.e. 0.01% for R45** **- if also the SCLs for acute toxicity should be translated to the CLP classification (as proposed in left column)** **- if R33 should be deleted and covered by an addition of Note H** **- if R33 should be changed ot T;R48/23/25, which should translate to STOT Rep. 1** **After FUII:** **BE: - how does Note H translate in CLR?** - agree [on SCL 0,01% for R45] for cadmium diformate as this chemical is soluble - SCLs for acute toxicity [are] useless as the specific concentration limits are intended for mixtures and in CLR, the later will be classified according to the additivity formula for acute toxicity - agrees with the ECB reasoning [for R33]. If the chemical would have been classified in CLR, it would have been most probably assigned STOT Rep 1. **DE: R33 crossreading including SCL; there was no consensus on cross-reading on other hazard classes than carcinogenicity at the meeting. So … to be consistent would not to translate the R33 into STOT Rep. 1 but to add Note H for all Cd compounds under discussion.** ECB Conclusion: There are no objections to addition of Note H. There is no strong support for changing R33 into T; R48/23/25. As Note H is already considered appropriate, it can also cover R33 (STOT). There is support for the SCL for carcinogenicity and no objections, a SCL 0,01% is therefore added. **⇨ Next ATP**

Cadmiumhexafluorosilicate(2); Cadmium fluorosilica **Index No: 048-005-00-7** **CAS No: 17010-21-8** **EC No: 241-084-0** **Classification:** Carc. Cat. 2; R45 *Agreed 0907* T; R23/25 *ATP29* ~~R33~~ (covered by note H) N; R50-53 *ATP29* **Note H** *Currently classified in Annex I (ATP 29): T; R23/25-R33-Xn; R68-N; R50-53* *Specific Concentration Limits(ATP 29):* *C ≥ 25 %: T, N; R23/25-33-50/53-68* *10 % ≤ C < 25 %: T, N; R23/25-33-51/53-68* *2,5 % ≤ C < 10 %: Xn, N; R20/22-33-51/53-68* *1 % ≤ C < 2,5 %: Xn; R20/22-33-52/53-68* *0,25 % ≤ C < 1 %: Xn; R20/22-33-52/53* *0,1 % ≤ C < 0,25 %: Xn; R20/22-33* ~~Specific Concentration Limits:~~ ~~C ≥ 25 %: T, N; R45-23/25-33-50/53~~ ~~10 % ≤ C < 25 %: T, N; R45-23/25-33-51/53~~ ~~2,5 % ≤ C < 10 %:T, N; R45-20/22-33-51/53~~ ~~1 % ≤ C < 2,5 %: T; R45-20/22-33-52/53~~ ~~0,25 % ≤ C < 1 %: T; R45-20/22-33-52/53~~ ~~0,1 % ≤ C < 0,25 %: T; R45-20/22-33~~ **Labelling:** T; N R: 45-23/25-33-50/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Carc. 1B; H350 Acute Tox. 3; H331* Acute Tox. 3; H301* Aquatic Acute 1; H400 Aquatic Chronic 1; H410 *Necessary to check the data for	Preparing the corrigendum of Annex I it was reported that the classification of 4 cadmium compound entries in Annex I classified with Xn; R68 should be changed into Carc. Cat. 3; R40. In *March 2006* TC C&L confirmed that this had been the intention but that the classifications should be re-discussed based on the classification in category 2 for carcinogenicity made for some other cadmium compounds listed in the draft 30th ATP list. *Follow-up of March 2006:* S**: At the March meeting a corrigendum of Annex I for four cadmium compounds was proposed. Some Cd compounds have already been updated with labelling for cancer which was also proposed at the meeting but the classification for reprotoxic effects has not been addressed. A read-across (if studies are not available) should also be made to reproductive toxicity in line with the already updated entries in Annex I with Repr. Cat. 2; R60/61 or Repr. Cat. 3; R62/63.** DK **agrees upon read-across regarding reproductive toxicity.** In *October 2006* it was agreed by the TC C&L that read across in principle would be possible, but that it should be examined more carefully for which endpoints. **DE** provided a table with solubility data for cadmium compounds (ECBI/61/06 Add. 2). *The TC C&L was asked to provide their opinions in written within the deadlines of the September meeting on the issue for which of the endpoints read across could be applied.* No further comments/positions were received. ***In September 2007*** the TC C&L agreed to revise this entry and classify it with Carc. Cat. 2; R45 (Carc 1B H350) based on the solubility data, which indicated that the compound was easily soluble. The toxicity would then be due to the presence of Cadmium ions and it was correct to classify this substance as other easily soluble Cadmium compounds already listed in Annex I. It was further agreed not to read across any other end-points such as for reproductive toxicity at this point in time, but in

confirmation of the classification. **Specific Concentration Limit:** ~~**ECB proposes:**~~ ~~**[C ≥ 10 %: Acute Tox 3* H301, H331**~~ ~~**0,1 % ≤ C <10%: [STOT Rep. 2, H373 ?] ;**~~ ~~**Acute Tox 4* H302, H332**~~ ~~**0,01 % ≤ C <0.1%: Carc 1B H350]**~~ **Carc 1B H350:C ≥ 0,01 %**	case a Member State had such a concern they should provide ECHA with an Annex XV dossier for re-discussion in the future. **After FUI:** **DE:** As only R45 has been crossread should not the entry be assigned Note H? **NL**: It was decided to classify for R45 based on read-across but not to read-across for other endpoints for practical reasons. As classification for other endpoints based on read-across cannot be excluded it is proposed to add note H. **ECB** will ask MS to react if they do NOT agree to add Note H. DE**: To agree on SCL according to CLaP may be very difficult in a written procedure. As only R45 was added, it could be discussed that the 0.01% SCL for the highly soluble Cd compounds should be crossread also.** ECB **will ask MS if the SCL of 0.01 % for R45 will be read across from the other highly soluble Cd compounds in Annex I.** **For SCL setting under CLaP, there may be no easy consensus for acute toxicity SCL [I would delete them as the CLaP formula in 3.1.3.6.1 automatically sets SCL because the LD/LC_{50} is included in the calculation; if not human data indicate necessity to deviate].** ECB **suggests to "translate" the SCLs also for acute toxicity to Annex VI for the time being in order not to loose this important hazard information, meanwhile the "issue" with the ATE formula in the GHS is being considered (the GHS formula does not take into account a SCL as currently in the EU).** **STOT SCL (R33) could be crossread as there are also SCL re-agreed on R48 for Cd sulphate and Cd chloride in the 29.ATP (ECB: 0.1% is already ascribed the entry for R33 and R20/22).** ECB**: ECB has looked at the other entries in Annex I of soluble Cd compounds (e.g. the Cd chloride, Cd fluoride, Cd sulphate) which all are classified with T; R48/23/25 (that will be translated into STOT Rep 1 H372). However, if R33 will be kept for this easily soluble compound (see above) an inconsistency with regard to the other soluble Cd compounds (will remain in Annex I and) will be introduced in Annex VI to the GHS CLP Regulation, since R33 is suggested to be translated into STOT Rep. 2.**

	ECB doubts that this inconsistency is justified in view of the well-known, documented and typical effects of Cd on e.g. the kidney and bone changes/damages also demonstrated in humans, caused by the Cd-ion after repeated exposure. These effects observed in humans (and animals), would rather justify STOT Rep. 1. **Of the reasons above, ECB suggests either to delete R33 and include Note H or to change R33 to R48/23/25, which should translate to STOT Rep. 1 H372.** ECB**: In summary, MS are asked during the FU to react:** **- if you do NOT agree to Note H to be assigned the entry in accordance with the above proposal from DE and NL to allow consideration of classification also for other endpoints** **-if the SCL should be cross read from the highly soluble Cd compounds, i.e. 0.01% for R45** **- if also the SCLs for acute toxicity should be translated to the CLP classification (as proposed in left column)** **- if R33 should be deleted and covered by an addition of Note H** **- if R33 should be changed to T;R48/23/25, which should translate to STOT Rep. 1** **After FUII:** **BE: - how does Note H translate in CLR?** - agree [on SCL 0,01% for R45] for cadmium diformate as this chemical is soluble - SCLs for acute toxicity [are] useless as the specific concentration limits are intended for mixtures and in CLR, the later will be classified according to the additivity formula for acute toxicity - agrees with the ECB reasoning [for R33]. If the chemical would have been classified in CLR, it would have been most probably assigned STOT Rep 1. **DE: R33 crossreading including SCL; there was no consensus on cross-reading on other hazard classes than carcinogenicity at the meeting. So … to be consistent would not to translate the R33 into STOT Rep. 1 but to add Note H for all Cd compounds under discussion.** ECB Conclusion: There are no objections to addition of Note H. There is no

	strong support for changing R33 into T; R48/23/25. As Note H is already considered appropriate, it can also cover R33 (STOT). There is support for the SCL for carcinogenicity and no objections, a SCL 0,01% is therefore added. ⇨ **Next ATP**
Cadmium iodide **Index No: 048-007-00-8** **CAS No: 7790-80-9** **EC No: 232-223-6** **Classification:** Carc. Cat. 2; R45 *Agreed 0907* T; R23/25 *ATP29* ~~R33~~ (covered by note H) N; R50-53 *ATP29* **Note H** *Currently classified in Annex I (ATP 29): T; R23/25-R33-Xn; R68-N; R50-53* *Specific Concentration Limits(ATP 29):* *C ≥ 25 %: T, N; R23/25-33-50/53-68* *10 % ≤ C < 25 %: T, N; R23/25-33-51/53-68* *2,5 % ≤ C < 10 %: Xn, N; R20/22-33-51/53-68* *1 % ≤ C < 2,5 %: Xn; R20/22-33-52/53-68* *0,25 % ≤ C < 1 %: Xn; R20/22-33-52/53* ~~Specific Concentration Limits:~~ ~~C ≥ 25 %: T, N; R45-23/25-33-50/53~~ ~~10 % ≤ C < 25 %: T, N; R45-23/25-33-51/53~~ ~~2,5 % ≤ C < 10 %: T, N; R45-20/22-33-51/53~~ ~~1 % ≤ C < 2,5 %: T; R45-20/22-33-52/53~~ ~~0,25 % ≤ C < 1 %: T; R45-20/22-33-52/53~~ **Labelling:** T; N R: 45-23/25-33-50/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Carc. 1B; H350	Preparing the corrigendum of Annex I it was reported that the classification of 4 cadmium compound entries in Annex I classified with Xn; R68 should be changed into Carc. Cat. 3; R40. In *March 2006* TC C&L confirmed that this had been the intention but that the classifications should be re-discussed based on the classification in category 2 for carcinogenicity made for some other cadmium compounds listed in the draft 30th ATP list. *Follow-up of March 2006:* S**: At the March meeting a corrigendum of Annex I for four cadmium compounds was proposed. Some Cd compounds have already been updated with labelling for cancer which was also proposed at the meeting but the classification for reprotoxic effects has not been addressed. A read-across (if studies are not available) should also be made to reproductive toxicity in line with the already updated entries in Annex I with Repr. Cat. 2; R60/61 or Repr. Cat. 3; R62/63.** DK **agrees upon read-across regarding reproductive toxicity.** In *October 2006* it was agreed by the TC C&L that read across in principle would be possible, but that it should be examined more carefully for which endpoints. **DE** provided a table with solubility data for cadmium compounds (ECBI/61/06 Add. 2). *The TC C&L was asked to provide their opinions in written within the deadlines of the September meeting on the issue for which of the endpoints read across could be applied.* No further comments/positions were received. ***In September 2007*** the TC C&L agreed to revise this entry

Acute Tox. 3; H331* Acute Tox. 3; H301* Aquatic Acute 1; H400 Aquatic Chronic 1; H410 *Necessary to check the data for confirmation of the classification. **Specific Concentration Limits:** ~~**ECB proposes:**~~ ~~**[C ≥ 10 %: Acute Tox 3* H301, H331**~~ ~~**0,1 % ≤ C <10%: [STOT Rep. 2, H373 ?] ;**~~ ~~**Acute Tox 4* H302, H332**~~ ~~**0,01 % ≤ C <0.1%: Carc 1B H350]**~~ **Carc 1B H350:C ≥ 0,01 %**	and classify it with Carc. Cat. 2; R45 (Carc 1B H350) based on the solubility data, which indicated that the compound was easily soluble. The toxicity would then be due to the presence of Cadmium ions and it was correct to classify this substance as other easily soluble Cadmium compounds already listed in Annex I. It was further agreed not to read across any other end-points such as for reproductive toxicity at this point in time, but in case a Member State had such a concern they should provide ECHA with an Annex XV dossier for re-discussion in the future. **After FUI:** **DE**: As only R45 has been crossread should not the entry be assigned Note H ? **NL:** It was decided to classify for R45 based on read-across but not to read-across for other endpoints for practical reasons. As classification for other endpoints based on read-across cannot be excluded it is proposed to add note H. **ECB** will ask MS to react if they do NOT agree to add Note H. DE**: To agree on SCL according to CLaP may be very difficult in a written procedure. As only R45 was added, it could be discussed that the 0.01% SCL for the highly soluble Cd compounds should be crossread also.** ECB **will ask MS if the SCL of 0.01 % for R45 will be read across from the other highly soluble Cd compounds in Annex I.** **For SCL setting under CLaP, there may be no easy consensus for acute toxicity SCL [I would delete them as the CLaP formula in 3.1.3.6.1 automatically sets SCL because the LD/LC_{50} is included in the calculation; if not human data indicate necessity to deviate].** ECB **suggests to "translate" the SCLs also for acute toxicity to Annex VI for the time being in order not to loose this important hazard information, meanwhile the "issue" with the ATE formula in the GHS is being considered (the GHS formula does not take into account a SCL as currently in the EU).** **STOT SCL (R33) could be crossread as there are also SCL re-agreed on R48 for Cd sulphate and Cd chloride in the 29.ATP (ECB: 0.1% is already ascribed the entry for R33 and R20/22) .** ECB: **ECB has looked at the other entries in Annex I of**

	soluble Cd compounds (e.g. the Cd chloride, Cd fluoride, Cd sulphate) which all are classified with T; R48/23/25 (that will be translated into STOT Rep 1 H372). However, if R33 will be kept for this easily soluble compound (see above) an inconsistency with regard to the other soluble Cd compounds (will remain in Annex I and) will be introduced in Annex VI to the GHS CLP Regulation, since R33 is suggested to be translated into STOT Rep. 2. ECB doubts that this inconsistency is justified in view of the well-known, documented and typical effects of Cd on e.g. the kidney and bone changes/damages also demonstrated in humans, caused by the Cd-ion after repeated exposure. These effects observed in humans (and animals), would rather justify STOT Rep. 1. **Of the reasons above, ECB suggests either to delete R33 and include Note H or to change R33 to R48/23/25, which should translate to STOT Rep. 1 H372.** ECB**: In summary, MS are asked during the FU to react:** **- if you do NOT agree to Note H to be assigned the entry in accordance with the above proposal from DE and NL to allow consideration of classification also for other endpoints** **-if the SCL should be cross read from the highly soluble Cd compounds, i.e. 0.01% for R45** **- if also the SCLs for acute toxicity should be translated to the CLP classification (as proposed in left column)** **- if R33 should be deleted and covered by an addition of Note H** **- if R33 should be changed ot T;R48/23/25, which should translate to STOT Rep. 1** **After FUII:** **BE: - how does Note H translate in CLR?** - agree [on SCL 0,01% for R45] for cadmium diformate as this chemical is soluble - SCLs for acute toxicity [are] useless as the specific concentration limits are intended for mixtures and in CLR, the later will be classified according to the additivity formula for acute toxicity - agrees with the ECB reasoning [for R33]. If the chemical would have been classified in CLR, it would have been most probably assigned STOT Rep 1. **DE: R33 crossreading including SCL; there was no**

	consensus on cross-reading on other hazard classes than carcinogenicity at the meeting. So … to be consistent would not to translate the R33 into STOT Rep. 1 but to add Note H for all Cd compounds under discussion. ECB Conclusion: There are no objections to addition of Note H. There is no strong support for changing R33 into T; R48/23/25. As Note H is already considered appropriate, it can also cover R33 (STOT). There is support for the SCL for carcinogenicity and no objections, a SCL 0,01% is therefore added. **⇨ Next ATP**
C085 **Type 475 Special purpose fibres** **[Man-made vitreous (silicate) fibres with random orientation with the following composition (% given by weight): 57.5-59.1% SiO_2, 5.4-6.2% Al_2O_3, 10.5-12.1% B_2O_3, 9.1-10.3% Na_2O, 3.0-3.6% K_2O, 1.5-2.1% CaO, 0.2-0.5% MgO, 3.6-4.4% ZnO, 4.6-5.4% BaO, 0.55-0.85% F_2]** **Extracted from the current entry with Index No: 650-017-00-8** **(New index number to be allocated)** **CAS No: 65997-17-3?** **EC No: 266-046-0?** **Classification proposal:** Carc. Cat. 3; R40 *Agreed 1006* ~~Xi; R38~~ *Agreed 0306* Note A and R *Current classification (23*	**Carcinogenicity** *In March 2006* Member States were split whether the discussed fibres should be classified in category 2 or category 3 for carcinogenicity. It was further brought up for discussion whether the E-glass and the 475-special purpose fibres should be classified differently. This was to be further clarified during the Follow-up and in the preparation of the next meeting, so the TC C&L would be able to conclude the discussion at their next meeting. *In October 2006* the TC C&L agreed to have different entries for the 'Type 475 Special purpose fibres' and the 'E-glass fibres', because they were considered to be different and different classification categories would apply for carcinogenicity. TC C&L agreed to classify 'Type 475 Special purpose fibres' with Carc. Cat. 3; R40 while 'E-glass fibres' would remain with the current Carc. Cat. 2; R49 classification. → Final classification proposal agreed by TC C&L IND sent in ECBI/10/05 Add. 6 for identification of the substances to be covered by the two entries. Member States were invited to react in case they did not agree with the entries as identified. FR: The current index 650-017-00-8 also covers refractory ceramic fibres (RCF) and should therefore not be restricted to E-fibres. Besides, the current index 650-016-00-2 which is classified Carc. Cat. 3;

ATP): Carc. Cat. 2; R49 - Xi; R38 *Note A and R* **Labelling:** Xn R: 40 S: (2-)36/37-46 **Classification assigned in accordance with the CLP Regulation:** Carc. 2; H351 (GHS classification confirmed by FR)	R40 and could apply by default to 475-type fibres, is specific because of nota Q which allows exemption of the carcinogenic classification under certain circumstances. For these reasons, we propose to have the following entries: - To keep the current entries Index 650-017-00-8 and Index 650-016-00-2 as they are. - To create one additional entry for E-fibres (with a new index number) and one additional entry for 475-fibres (which will differ from index 650-016-00-2 by the absence of nota Q). Besides, the chemical composition of the glass may not be sufficient to characterise appropriately the entries. To our knowledge, E-glass may also be used in other type of glass fibres than special purpose fibres, such as continuous glass filaments for example. Therefore, an appropriate way to identify the entries could be to specify both composition and size and to limit the entries to fibres with a mean diameter of less than 3 µm. **IND** sent documents ECBI/10/05 Add. 8 parts I, II and III. The values of the type 475 fibers are corrected in correspondence with the table of document 10/05 Add. 8 part II. *MS were asked to react in written in case they do not agree to the new IND proposal prior 31 August 2007. In case no reactions no further detailed discussion is foreseen to take place at the September meeting, but the entry as defined here can be considered confirmed.* No further comments were received. **Final Conclusion:** TC C&L has then confirmed the entry as written here, and there will be no further discussion. **After FUII:** **ECB:** The CAS No 65997-17-3 is coupled to EC No 266-046-0 with the substance name *Glass, oxide, chemicals* and a description starting with '"This category encompasses the various chemical substances manufactured in the production of inorganic glasses……..". Whether the CAS and EC Nos should be assigned to the more specified entry *Type 475 Special purpose fibres* still has to be decided before this entry is included in the next ATP. ⇨ **Next ATP**
C085 **E-glass Special Purpose fibres** **[Man-made vitreous (silicate) fibres with random orientation with the following**	**Carcinogenicity** *In March 2006* Member States were split whether the discussed fibres should be classified in category 2 or category 3 for carcinogenicity. It was further brought up for discussion whether the E-glass and the 475-special purpose fibres should be classified

composition (% given by weight): 54–55% SiO_2, 14–15% Al_2O_3, 7 –8% B_2O_3, 0-0.6% Na_2O, 0-0.2% K_2O, 18–21% CaO, 0.3-3% MgO, 0.2-0.4% Fe_2O_3, 0-1% F_2, 0.5-0.6% TiO_2]~~[$T_{i2}O$, 18.3-24.8% MgO+CaO+Na_2O+K_2O+BaO]~~ **Index No: ~~650-017-00-8~~ (New index number to be allocated)** **CAS No: 65997-17-3?** **EC No: 266-046-0?** **Classification proposal:** Carc. Cat. 2 ; R49 ~~Xi; R38~~ *Agreed 0306* Note A and R *Current classification (23 ATP): Carc. Cat. 2; R49 - Xi; R38* *Note A and R* **Labelling:** T R: 49 S: 53-45 **Classification ~~and hazard statements~~ assigned in accordance with the CLP Regulation:** Carc. 1B; H350i (GHS classification confirmed by FR)	differently. This was to be further clarified during the Follow-up and in the preparation of the next meeting, so the TC C&L would be able to conclude the discussion at their next meeting. ***In October 2006*** the TC C&L agreed to have different entries for the 'Type 475 Special purpose fibres' and the 'E-glass fibres', because they were considered to be different and different classification categories would apply for carcinogenicity. TC C&L agreed to classify 'Type 475 Special purpose fibres' with Carc. Cat. 3; R40 while 'E-glass fibres' would remain with the current Carc. Cat. 2; R49 classification. → Final classification proposal agreed by TC C&L IND sent in ECBI/10/05 Add. 6 for identification of the substances to be covered by the two entries. Member States were invited to react in case they did not agree with the entries as identified. DE: Both E- and 475-glass fibres have a K_{NB} index greater than 18%. Carc. Cat. 3 classification therefore applies as a default. Thus, for R49 classification a separate entry is necessary for E-glass. Therefore it is not necessary to define a separate 475 glass entry, but only re-name the current 650-017-00-8 entry. **IND** sent documents ECBI/10/05 Add. 8 parts I, II and III. **IND** agrees in document ECBI/10/05 Add. 8 part I, to use table 2.15 from document ECBI/10/05 for the E-glass fibers. *MS were asked to react in written in case they do not agree to the new IND proposal prior 31 August 2007. In case no reactions no further detailed discussion is foreseen to take place at the September meeting, but the entry as defined here can be considered confirmed.* No further comments were received. **Final Conclusion:** TC C&L has then confirmed the entry as written here, and there will be no further discussion. **After FUII:** **ECB:** The CAS No 65997-17-3 is coupled to EC No 266-046-0 with the substance name *Glass, oxide, chemicals* and a description starting with '"This category encompasses the various chemical substances manufactured in the production

	of inorganic glasses……..". Whether the CAS and EC Nos should be assigned to the more specified entry *E-glass Special purpose fibres* still has to be decided before this entry is included in the next ATP. ⇨ **Next ATP**
I062 (IT) **Chlorodifluoromethane** **CAS No: 75-45-6** **EC No: 200-871-9** **Classification:** **NC for carcinogenicity** ***Agreed 0907*** **Repr. Cat. 3; R63** ***Agreed 0907*** **Labelling:** Xn R: 63 S: (2-)36/37-46 **Classification assigned in accordance with the CLP Regulation:** Repr. 2; H361d	*In preparation for the September 2007* meeting IT sent a C&L proposal (ECBI/136/06) for Chlorodifluoromethane. IT **submitted the annexes 1, 2 and 3 to their proposal ECBI/136/06 distributed as Add. 1, 2 and 3 with Revision 2 of the September agenda.** ***In September 2007*** it was agreed not to classify chlorodifluoromethane for carcinogenicity. **Several MS were of the opinion that the effects seen for reproductive toxicity were a borderline also because of the rather high dose levels where they occurred but as they were considered severe effects the TC C&L recommended to classify with Repr. Cat. 3; R63.** ⇨ **Next ATP**
F054 (F) 2-butoxyethylacetate 607-038-00-2 CAS: 112-07-2 **EC: 203-933-3** **Classification:** Xn; R21/22 *Agreed 0907* *Current classification (19 ATP): Xn; R20/21* **Labelling:** Xn R: 21/22	A new classification proposal was provided by FR in ECBI/50/07, circulated with Revision 3 of the September agenda. ***In September 2007*** **the** proposal submitted by FR to delete Xn; R20 and to add Xn; R22 for 2-butoxyethylacetate in Annex I was agreed. ⇨ **Next ATP**

S: (2-)36/37-46 **<u>Classification assigned in accordance with the CLP Regulation:</u>** **Acute Tox. 4; H312** **Acute Tox. 4; H302**	
A031(DE) **2-ethoxyethanol (stabilised)** **603-012-00-X** **CAS: 110-80-5** **EC: 203-804-1** **<u>Classification:</u>** R 10 Repr. Cat. 2; R60-61 *Agreed 0907* Xn; R20/22 *Agreed 0907* *Current classification (19 ATP): R10* *Repr. Cat. 2; R60-61 - Xn; R20/21/22* **<u>Labelling:</u>** T R: 60-61-10-20/22 **S: 53-45** **<u>Classification assigned in accordance with the CLP Regulation:</u>** Flam.Liq.3; H226 Repr. 1B; H360FD Acute Tox. 4; H332 Acute Tox. 4; H302	A new classification proposal was provided by **DE** in ECBI/48/07, circulated with Revision 3 of the September agenda. ***In September 2007*** the proposal submitted by DE to delete R21 for 2-ethoxyethanol in Annex I was agreed by the TC C&L. **After FUI**: **NL** comment that according to them it was concluded to add "stabilised" to the name of the substance. **ECB**: The TC C&L is asked to comment if "stabilised" was agreed at the meeting and/or should be added. **After FUII:** **DE:** the word stabilised is included in the proposal as was agreed at the meeting. **ECB:** 'stabilised' is included. ⇨ **Next ATP**
D151 (DE) **2,4,4-trimethylpentene** **CAS: 25167-70-8** **EC: 246-690-9**	A new classification proposal was provided by **DE** in ECBI/49/07, circulated with Revision 3 of the September agenda. ***In September*** *2007* R65 was agreed based on human data. ~~BE asked for more information on the phys-chem data (i.e. viscosity).~~ For skin and respiratory tract irritation it was

Classification: **F; R11** ***Agreed*** ***FU 0907*** **NC Xi; R37/38** ***Agreed 0907*** Xn; R65 *Agreed 0907* R67 [N; R50-53] **Labelling:** F, Xn[, N] R: 11-65-67[-50/53] **S: (2-)16-29-62-[60-61]** **Classification assigned in accordance with the CLP Regulation:** Flam. Liq. 2; H225 **Asp. Tox. 1; H304** **STOT Single 3; H336** **Agreed** **FU 09/07** [Aquatic Acute 1 H400] [Aquatic Chronic 1 H410]	agreed that the available data does not warrant classification with Xi; R38/37. ECB will evaluate whether to make a written procedure and ask the TC C&L Phys Chem and Environmental experts to agree on classification with F; R11 and N; R50-53, respectively. Else the partial classification concerning the environment should be handed over for discussion at ECHA with support of an Annex XV dossier. A proposal for 2 entries in Annex I was submitted by **DE** in ECBI/49/07 Add.1. **After FUI**: **DE**: S16 and S29 to be added in case F, R11 agreeable. S62 is obligatory and has to be added; then S46 is superfluous. **DE**: STOT Single 3 was requested by the TC C&L 09/07 (for Narcotic effects). **DE**: If this substance (CAS: 25167-70-8) will be included in the 1st ATP of CLaP without F, R11 and also the entry for the 'other' trimethylpentene CAS 107-39-1 will not be changed please add Note H. Otherwise the deviating classification for CAS 107-39-1 (both R 11 and different ENV classification) will be confusing. **After FUII** **BE:** agrees with the DE proposal for S-phrases and the STOT Cat 3 pertaining to the sedation effect. **NL:** Agree with inclusion of S16, S29 and S62 and removal of R46. STOT single 3 was concluded **ECB:** By agreeing to add S16 and S29 MS indirectly say that F; R11 is agreeable. This is also in line with the data in the RAR. R67 is assigned as well, in analogy with the STOT Single 3 H336 classification. **ECB conclusion**: Health classification and classification for flammability are concluded. No written procedure has been made for environmental classification, which therefore has to be further discussed. **⇨ Hand-over to ECHA**
A016 (DE) **Nitrobenzene** **609-003-00-7**	A new classification proposal was provided by DE in ECBI/38/07, circulated with Revision 2 of the September agenda.

CAS: 98-95-3 **EC: 202-716-0** **Classification:** Carc. Cat. 3; R 40 *Agreed 0907* Repr. Cat. 3; R 62 *Agreed 0907* T; R 23/24/25 *Agreed 0907* T; R 48/23/24/25 *Agreed 0907* N; R51-53 *ATP22* *Current classification (22 ATP): Carc. Cat. 3; R40- Repr. Cat. 3; R62 T; R23/24/25-48/23/24 - N; R51-53* **Labelling:** T, N R: 23/24/25-40-48/23/24/25-62-51/53 **S: (1/2-)28-36/37-45-61** **Classification ~~and hazard statements~~ assigned in accordance with the CLP Regulation:** Carc. 2; H 351 Repr. 2; H361f Acute Tox. 3; H331 Acute Tox. 3; H311 Acute Tox. 3; H301 STOT Rep. 1; H372	***In September 2007* the** proposal submitted by DE to add Xn; R48/25 to the current classification in Annex I was agreed by the TC C&L. **⇨ Next ATP**
D116 (DE) **Vinylacetate** **607-023-00-0** **CAS: 108-05-4** **EC: 203-545-4** **Classification:** F; R11 Carc. Cat. 3; R40 *Agreed 0907*	A new classification proposal was provided by **DE** in ECBI/39/07, circulated with Revision 2 of the September agenda. **DE** updated their proposal with ECBI/39/07 Rev. 1 (Annex XV dossier). ***In September** 2007* the proposal submitted by DE was agreed. There was a short discussion on a possible classification for mutagenic effects. It was considered a borderline case but the TC C&L agreed not to classify vinylacetate for this endpoint.

Xn; R20 *Agreed 0907* Xi; R37 *Agreed 0907* *Current classification 19 ATP): F; R11* **Labelling:** F; Xn R: 11-20-37-40 S(: 2-)16-~~23~~-33-36/37 **Classification assigned in accordance with the CLP Regulation:** Flam. Liq. 2; H225 Carc. 2; H351 Acute Tox 4; H332 STOT Single 3; H335	**After FUI**: **DE** suggests to delete S23 **After FUII: S23 deleted since there are no objections to this.** ⇨ **Next ATP**
D135 (DE) **4-tert-butylbenzoic acid** **CAS: 98-73-7** **EC: 202-696-3** **Classification:** Repr. Cat. 2; R60 *Agreed 0907* Xn; R22 *Agreed 0907* T; R48/23/24/25 *Agreed 0907* N; R51-53 *Agreed 09/05* **Labelling:** T, N R: 60-22-48/23/24/25-51/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 1B; H360F Acute Tox 4; H302 STOT Rep. 1; H372 Aquatic Chronic 2; H411	A new classification proposal was provided by **DE** in ECBI/40/07, circulated with Revision 2 of the September agenda. ***In September 2007*** the TC C&L agreed that for acute toxicity the available LC50 for inhalation and the LD50 for dermal application were not sufficient for classification. Only the oral route was then recommended for classification (R22). Repr. Cat. 2; R60 and T; R48/23/24/25 were agreed on the basis of the DE proposal without discussion ⇨ **Next ATP**

D137 **Entry 1:** **Dibutyltin di(acetate)** **Not listed in Annex I** **CAS No: 1067-33-0** **EC No: 213-928-8** **Classification:** Repr. Cat. 2; R60-61 *Agreed 1105* Muta. Cat. 3; R68 *Agreed 1105* Xn; R22 *Agreed 1105* C; R34 *Agreed 1105* T; R48/25 *Agreed 1105* N; R50-53 *Agreed 0107* M=10 **Labelling:** T, N R: 60-61-22-34-41-48/25-68-50/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 1B; H360FD Muta. 2; H341 Acute Tox. 4; H302 Skin Corr. 1B; H314 STOT Rep. 1; H372 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M=10 **Entry 2:** **Dibutyltin dilaurate** **Not listed in Annex I** **CAS No: 77-58-7** **EC No: 201-039-8** **Classification:**	*In November 2005* the TC C&L agreed in general to the classification of dibutyltin di(acetate) as proposed by IND but in addition C; R34 (rather than Xi; R38) and Muta. Cat. 3; R68 should apply. *In October 2006* IND suggested to put Specific Concentration Limits based on the dibutyltin content in the dibutyltin compounds on the basis of the reasoning presented in ECBI/25/05 Add. 5. ECB interpret the suggestion that in this case a 1% limit could be applied for reproductive toxicity instead of 0.5%. NL suggests to set lower SCL than suggested by IND or to keep the general concentration limits as defined in the Preparations Directive. They explain their position in detail in document ECBI/25/05 Add. 7. DK do not agree to set specific concentration limits at this stage. We should await the potency discussions and its implications for setting specific concentration limits for reproductive toxicity end-points. IND provided further information requested by NL for setting of SCL of dibutyltin compounds in ECBI/25/05 Add. 8. BE: Concerning the developmental effects, we completely agree with the SCLs proposed by NL, there is a sufficient amount of reproducible data from different laboratories to ground our scientific judgement. However, according to us, it is more difficult to set SCL for fertility. In the GLP-TNO study, the NOAEL for fertility is 2 mg/kg but the NOAEL for general toxicity is only 0.3-0.4 mg/kg. In the Ema and Harazono study, where DBTCl was administered on GD 0-3 or on GD 4-7, the fertility does not seem to be more adversely affected than the development. We found reference to another study on dibutyltin diacetate in a WHO document (Dialkyltins in Drinking-water – Background document for development of WHO Guidelines for Drinking-water quality) (WHO/SDE/WSH/03.04/109). In this study of Noda T et al (1988), DBTC was given to pregnant wistar rats on days 0-19 of pregnancy at 0, 1.7, 5.0 or 15 mg/kg of body weight. It would be interesting to see if fertility parameters are reported in this study and if the NOAEL is in the same range as for the Ema and Harazono study. In conclusion, we do not think we have enough data on fertility allowing to lower the SCL for this endpoint. But if the majority of MS is convinced that fertility is a more sensitive indicator of toxicity than developmental effects, then we need to go back to the entry of Dibutyltin chloride (30th ATP) and propose SCL for these effects.

Repr. Cat 2; R60-61 *Agreed 1105* Muta. Cat. 3; R68 *Agreed 1105* Xn; R22 *Agreed 0306* Xi; R38 *Agreed 0306* T; R48/25 *Agreed 1105* N; R50-53 *Agreed 0107* **Labelling:** T, N R: 60-61-22-38-48/25-68-50/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 1B; H360FD Muta. 2; H341 Acute Tox. 4; H302 Skin Irrit. 2; H315 STOT Rep. 1; H372 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 **Entry 3:** **Dibutyltin maleate; 2,2-dibutyl-(1,3,2-dioxastannepin-4,7-dione)** **Not listed in Annex I** **CAS No: 78-04-6** **EC No: 201-077-5** **Classification:** Repr. Cat 2; R60-61 *Agreed 1105* Muta. Cat. 3; R68 *Agreed 1105* T; R23-48/25 *Agreed 1105* Xn; R22 *Agreed 1105* Xi; R36	IND (Kaneka) sent document ECBI/25/05 Add. 10, disagreeing with the proposal by NL for lower specific concentration limits for the dibutyltin compounds. IND (Etinsa) sent document ECBI/25/05 Add. 11 agreeing to a pragmatic approach to apply general concentration limits (GCLs). Detailed calculations for individual substances, deviating from the GCLs, should be on basis of data provided by specific industry sectors. ECB proposes to use general concentration limits for the dibutyltin compounds, since the specific concentration limits as calculated by NL are very close to the general concentration limits. *MS were asked to react in case this is not supported.* **NL:** We agree with the use of GCL for most dibutyltin compounds. **IND** (Kaneka) provided document ECBI/25/05 Add. 12 circulated with Revision 2 of the September agenda. Pointing out that the dubutyltin compound of most interest for them and giving significant difference in SCL calculated based on the dibutyltin moiety, is Dibutylbis(pentane-2,4-dionate-O'O')tin (CAS: 22673-19-4, EC: 245-152-0). They suggest to add this substance to Annex I and set the SCL to 0.54% with a note that this SCL is set by reference to the concentration of the dibutyltin moiety. **IND** (Kaneka) sent a revision of ECBI/25/05 Add. 12, which was linked to the 4th revision of the agenda. *MS were asked to react in written within the deadlines of the September meeting to whether they can agree or strongly oppose to the IND proposal.* **NL:** For dibutylbis(pentane-2,4-dionate-O'O')tin, we agree with a SCL of 0.54% for R61. For R60, it should be discussed whether the available data are sufficient for determining a SCL but we do not strongly oppose this SCL. General comment: General Concentration Limits under the new Regulation: C ≥ 0.3% : Repr. 1B – H360FD GHS classifications indicated with * need to be checked with data. ***In September 2007*** the TC C&L agreed that general

Agreed 1105 N; R50-53 *Agreed 0107* **Labelling:** T, N R: 60-61-22-23-36-48/25-68-50/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 1B; H360FD Muta. 2; H341 Acute Tox. 23; ~~H331*~~H330 Acute Tox. 4; H302 Eye Irrit. 2; H319 STOT Rep. 1; H372 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	concentration limits would apply to the first 5 entries for dibutyltin compounds as listed here. They also agreed to further introduce the entry 6 for Dibutylbis(pentane-2,4-dionate-O,O')tin into Annex VI of the CLP Regulation. This entry would not be indicated with specific concentration limits under the current classification system, but under the classification in accordance with the CLP Regulation a specific concentration limit would be set to 0.5%, i.e. equal to the current general limit. **After FUII:** **NL**: Dibutyltin di(acetate) Acute Tox 4 H302 is correct (substance specific data). STOT Rep. 1 H372 is correct (read-across from DBT-diCl Dibutyltin dilaurate Acute Tox 4 H302 is correct (substance specific data, males). STOT Rep. 1 H372 is correct (read-across from DBT-diCl) Dibutyltin maleate:- Acute Tox 4 H302 is correct (substance specific data). Acute Tox 3 H331 should be Acute Tox 2 H330 (LC50=0.317 mg/l) STOT Rep. 1 H372 is correct (read-across from DBT-diCl) Dibutyltin oxide:- Acute Tox 3 H331 is not correct because R25 is oral. This should be Acute Tox 3 H301 (substance specific data). STOT Rep. 1 H372 is correct (read-across from DBT-diCl) ⇨ **Next ATP**
Entry 4: **Dibutyltin oxide** **Not listed in Annex I** **CAS No: 818-08-6** **EC No: 212-449-1** **Classification:** Repr. Cat 2; R60-61 *Agreed 1105* Muta. Cat. 3; R68 *Agreed 1105* T; R25-48/25 *Agreed 1105* Xi; R41 *Agreed 1105* N; R50-53 *Agreed 0107* **Labelling:** T, N R: 60-61-25-41-48/25-68-50/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 1B; H360FD	

Muta. 2; H341 Acute Tox. 3; ~~H331*~~H301 Eye Dam. 1; H318 STOT Rep. 1; H372 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 **Entry 5:** **Dibutyltin salts with the exception of those specified elsewhere in this Annex** **Not listed in Annex I** **Classification:** Repr. Cat 2; R60-61 *Agreed 1105* Muta. Cat. 3; R68 *Agreed 1105* T; R48/25 *Agreed FU 1105* N; R50-53 *Agreed 0107* Note H **Labelling:** T, N R: 60-61-68-50/53 S: 53-45-60-61 **Classification assigned in accordance with the CLP Regulation:** Repr. 1B H360FD Muta. 2 H341 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 Note H **Entry 6:** **Dibutylbis(pentane-2,4-dionate-O,O')tin** **Not listed in Annex I** **Cas No: 22673-19-4** **EC No: 245-152-0** **Classification:**	

Classification assigned in accordance with the CLP Regulation: Ox. Gas 1; H270 Acute Tox. 2; H330 Eye Irrit. 1; H318 STOT Single 3; H335 Skin Irrit. 2; H315 Aquatic Acute 1; H400 M=100	**experience was not supported. TC C&L agreed to recommend classification with Xi; R 37/38-41.** ECB has adjusted the S-phrases in accordance with the classification agreed at the meeting. **After FUI:** **UK** repeated their suggestion that T+; R26 may be more appropriate based on findings from individual studies and do not support the IT proposal for T; R23 and their approach to pool LC50 values obtained from several studies (see ECBI/134/06 Add. 1). **ECB:** The TC C&L are asked if T+; R26 is supported on the basis of the studies mentioned by UK in ECBI/134/06 Add. 1. **After FUII:** **BE:** Whereas we could agree with UK that pooling method is not usesd in our today's procedure for acute toxicity classification, there are no new data since the last C&L (1980!) and no convincing argument that a change in classification is needed. **DE:** The proposal sent in by UK does not contain precise information on the additional acute inhalation tests which are not included in the Italian dossier. E.g. it is not given with which exposure times these tests were performed nor is it given which time extrapolation factors were used to extrapolate to 4h exposures. So it is not possible to decide whether R23 or R26 may be more appropriate. **NL:** agree with the proposal for R26. **IE:** The Irish CA agrees with the UK that the LC50 determinations should not be based upon pooled data, and that the study by Zwart and Wouterson cited by the UK is an example of a single, good quality, comprehensive study upon which it would be appropriate to base LC50 extrapolations. Whilst the IT proposal references a 30 minute LC50 of 300-400ppm (0.9-1.2 mg/l) derived from pooled data (UK Toxicity Working Party of Major Assessment Panel, 1985), the value used to derive the 4hr LC50 appears to be from a single study conducted in rats. Indeed, in the C&L proposal prepared by IT, the extrapolated LC50(rat) at 4 hours is calculated to be 0.65mg/l based upon a LC50(rat) of 1.344mg/l at 1 hour, which is similar to the LC50(rat) obtained by Zwart and Wouterson (1.319mg/l) after a 60 minute exposure. A 4 hour LC50 estimate calculated from the Zwart and Wouterson data using the equation $C^n.t=k$, (where n=2) derives an value of 0.659mg/l which is in accordance with the value calculated by IT and not the value of 0.35mg/l calculated by the UK from the same data.

	The Irish CA considers that the 4 hour LC50 value calculated by IT (0.65mg/l) is valid and that the C&L proposal of T: R23 is correct. **ECB conclusion:** Based on the comments in FUII the classification for acute toxicity will remain T; R23 the classification according to the CLP Regulation will be Acute Tox. 2; H330. ⇨ **Next ATP**

1.2 3 SUBSTANCES FOR WHICH CLASSIFICATION AND LABELLING FOR HEALTH EFFECTS WILL BE FORWARDED TO ECHA

U065 (UK) **Styrene** **601-026-00-0** **CAS No: 100-42-5** **EC No: 202-851-5** **Classification:** **R10** **NC for carcinogenicity** ***Agreed 0907*** **NC for mutagenicity** ***Agreed 0907*** **[Repr. Cat. 2; R61/Repr. Cat. 3; R63/NC]** **Xn; R20-48/20** ***Agreed 0907*** **Xi; R36/37/38** ***Agreed 0907*** *Current classification (19 ATP): R10-Xn; R20-Xi; R36/38 SCL: C ≥ 12,5 % Xn; R20-36/38* **Labelling:** Xn [T] R: 10-20-36/37/38-48/20-[61/63]	**Respiratory tract irritation** In *March 2006* the TC C&L agreed to add R37 to the current classification and to delete specific concentration limits in accordance with the UK proposal in ECBI/19/06. At *TCNES IV in 2005* some MS (S, DK, N and A) had concerns for mutagenicity. Also a discussion of this endpoint based on a UK proposal was then foreseen. TC C&L agreed to postpone the discussion to the next meeting to include also the basis for agreement on classification/no classification for mutagenicity. **IND** submitted document ECBI/19/06 Adds. 5, 6, 7, 8 and 9, including comments on the Carcinogenicity, Mutagenicity, Reprotoxicity and R48 classification proposals. Add. 9 includes a scientific paper on human lung tumour formation. These documents were distributed with Rev. 2 to the September agenda. **DK** sent in a classification proposal ECBI/19/06 Add. 10 circulated with Revision 2 of the September agenda including in addition to the classification proposed by the rapporteur further classification with Xn; R48/20; Carc. Cat. 3 R40; Mut Cat. R68; Rep. Cat. 2 R61. ***In September 2007*** it was agreed not to classify styrene for carcinogenicity and mutagenicity**.** **Repeated dose effects:** For repeated dose effects it was agreed to classify with Xn;

[S: (2)-26-36/37] No specific concentration limits. **Classification assigned in accordance with the CLP Regulation:** Flam. Liq. 3; H226 [Repr. 1B; H360D/Repr. 2; H361d] Acute Tox. 4; H332 Eye Irrit. 2; H319 STOT Single 3; H335 Skin Irrit. 2; H315 STOT Rep. 1; H372	R48/20 on basis of ototoxicity observed in animals at concentrations above the criteria but supported by findings in humans. Under GHS this would be STOT Rep. 1. **Reproductive toxicity:** No agreement could be reached. The DK proposal for Cat. 2 R61 was supported by SE, 7 of the present MS experts were in favour of Cat. 3; R63, and 10 of the present MS experts preferred no classification. 1 MS expert did not have an opinion. Several MS experts expressed that they did not have enough experience with developmental neurotoxicity effects and therefore it was difficult to decide on basis of the available data whether the effects were specific. DK will substantiate their proposal and send this information prior 7 November to allow for reactions during the second Follow-up period. ECB promised to check on which dose levels for adverse effects the risk characterisation was based on and also this information would be available in the follow-up period. MS changing their position from the one expressed at the meeting or MS not present at the meeting are then asked to react during FU II. It was considered most probable that this substance should be handed over to ECHA for further discussion on reproductive toxicity. However a final decision on this would be made only at the end of the Follow-up period. **ECB** checked the wording in the RAR (summary of effects on reproduction): "Overall, it can be concluded that styrene does not cause developmental toxicity in animals as evaluated by structural endpoints at inhalation exposures of up to 600 ppm and oral exposures of up to 250 mg/kg/day and by neurological endpoints at inhalation exposures of up to 500 ppm. However, reduced pup growth and pup developmental delays (delays in attaining some pre-weaning developmental landmarks and in acquiring preputial separation, decreased swimming ability, slight shift in the normal pattern of motor activity and small reductions in forelimb grip strength) were seen postnatally in rats at exposure levels (300-500 ppm) causing, in some cases, maternal toxicity." **After FUI:** **UK** re-iterate their position that there is no reliable evidence that styrene causes specific developmental neuro toxicity and do not agree with a classification for developmental toxicity (ECBI/19/06 Add. 10)

	After FUII: **DK** re-iterate their position in response to the UK comments (ECBI/19/06 Add. 12) stating that the effect on grip strength is reliable data along with evidence of other developmental delays and that this warrants classification for developmental toxicity. **NL** agree with no classification for developmental toxicity. **NO:** Even if only one of the studies described in ECBI/19/06 Add. I part V Annex IV is OECD- and GLP-compliant other studies support the results obtained in this study. It can, however, not be excluded that the reduced grip strength observed after exposure to 500 ppm of styrene is due to the reduced body weight of the F_2 pups. We therefore suggest that styrene should be classified Repro, Category 3; R63 Possible risk to the unborn child. Document ECBI/19/06 Add. 13 attached in file **13** PL: **THERE ARE NO EVIDENCE WHICH ALLOW TO CLASSIFY STYRENE AS A DEVELOPMENTAL TOXICANT JUSTIFY CATEGORY 1 OR 2. HOWEVER, THERE ARE SOME EVIDENCE OF THE LOW DEGREE DEVELOPMENT DELAY OF THE OFFSPRING OF EXPOSED TO STYRENE PARENTAL ANIMALS WHICH NOT EXCLUDE A POSSIBILITY OF THE DEVELOPMENTAL IMPAIRMENT IN HUMAN. IT IS PROPOSED TO CLASSIFY STYRENE AS A DEVELOPMENTAL TOXICANT IN CATEGORY 3 (SUBSTANCES WHICH CAUSE CONCERN FOR HUMANS OWING DEVELOPMENTAL TOXIC EFFECTS). DOCUMENT ECBI/19/06 ADD. 14 ATTACHED IN FILE** **IRL:** accepts the arguments proposed by Rapporteur (UK) and IND that Repr. Cat 2: R61 is not justified. [ECB: IRL doesn't comment on Repr. Cat. 3; R63]. **ECB conclusion:** There is no agreement on the classification for developmental effects. **⇨ Hand-over to ECHA**
C067(F) **Chloroform (Trichloromethane)** **602-006-00-4** **CAS: 67-66-3** **EC: 200-663-8** **Classification:** **Carc. Cat. 3; R40** ***Agreed 0907***	A new classification proposal was provided by FR in ECBI/42/07, circulated with Revision 2 of the September agenda. ***In September 2007*** TC C&L agreed not to classify chloroform with Xi; R37 as the nasal effects reported were rather covered by Xn; R48/20. Further TC C&L agreed that R48/22 could be deleted as effects were only seen at high doses. They also agreed on classification with Repr. Cat. 3; R63 based on the FR proposal. The narcotic effects that would be covered by Xn; R20 under

[Muta Cat. 3; R68] **Repr. Cat. 3; R63** ***Agreed 0907*** **Xn; R20/22-48/20** ***Agreed 0907*** **NC Xn; R48/22** ***Agreed 0907*** **Xi; R36/38** ***Agreed 0907*** **NC Xi; R37** ***Agreed 0907*** **NC for the ENV** ***Agreed 0107*** *Current classification (19 ATP): Xn; R22-48/20/22 - Xi; R38 - Carc. Cat. 3; R40* **Labelling:** Xn R: 20/22-36/38-40-48/20-63-[68] **S: (2-)36/37-46** **Classification assigned in accordance with the CLP Regulation:** **Carc. 2; H351** **[Muta. 2; H341]** **Repr. 2; H361d** **Acute Tox. 3; H331** **Acute Tox. 4; H302** **STOT Rep. 2; H373** **Eye Irrit. 2; H319** **Skin Irrit. 2; H315** **STOT Single 3; H336**	the current system would trigger classification with STOT Single 3 under the CLP Regulation. **Mutagenicity:** No agreement could be reached on mutagenicity. 5 of the present MS experts were in favour of Muta. Cat. 3: R68, 10 experts preferred no classification and 4 experts did not have a final position. FR will revise their proposal with more justification for Muta. Cat. 3 R68 and provide this to the ECB prior 7 November. MS changing their position from the one expressed at the meeting or MS not present at the meeting are then asked to react during FU II. A final decision whether the discussion on mutagenicity must be handed over to ECHA will be made only at the end of the Follow-up period. ECB has updated the S-phrases in accordance with the classification agreed at the meeting (i.e. added S46). Comments with a proposal for Muta. Cat. 3; R68 were sent by **SE** in ECBI/42/07 Add.1. A new proposal for Muta Cat. Cat. 3; R68 was submitted by **FR** after TCNES discussion in ECBI/42/07 Add.2. **After FUI:** Mutagenicicity **DE** still supports R68. **FR** provided further additional information to determine whether chloroform is an *in vivo* mutagen and should be classified as Muta. Cat. 3; R68 (ECBI/42/07 Add. 3). **ECB**: On the bases of the additional information on mutagenicity provided by **FR** (ECBI/42/07 Add. 3), MS especially those who have changed their position from the one put forward at the TC C&L meeting or who were not present at the meeting are welcome to react during FUII. **After FUII:** **NL:** agrees with Muta Cat. 3 R68 **IRL**: has considered the summary data presented in this document and we believe that there is insufficient evidence to classify chloroform as Mut. Cat 3: R68. Many of the positive effects seen appear to be species specific, and appear to be mediated by cyp450 metabolism to phosgene in certain target organs. Despite these results the overwhelming body of evidence is negative and on this basis we considered that chloroform should not be classified. **ECB/FR: Dec 2007 TECNES meeting decided that further**

	testing for mutagenicity is necessary before any conclusion can be drawn. **⇨ Hand-over to ECHA**
Q010 **Vanadium pentoxide** **Index No: 023-001-00-8** **CAS No: 1314-62-1** **EC No: 215-239-8** **Classification:** [Carc. Cat. 2; R45] [Muta. Cat. 2; R46] Repr. Cat. 3; R63 *ATP 25* Xn; R20/22 *ATP 25* T; R48/23 *ATP 25* Xi; R37 *ATP 25* N; R51-53 *ATP 25* *Currently classified in Annex I (ATP 25): Muta. Cat. 3; R68 - Repr. Cat. 3; R63 - T; R48/23 - Xn; R20/22 - Xi; R37 - N; R51-53* **Labelling**: T, N R : [45-46-] 20/22-37-48/23-63-51/53 S : [53-45] **Classification and hazard statements assigned in accordance with the CLP Regulation:** [Carc. 1B; H350] [Muta. 1B; H340] Repr. 2; H361d Acute Tox. 4; H332* Acute Tox. 4;H302* STOT Rep. 1; H372i STOT Single 3; H335 Aquatic Chronic 2; H411 * Necessary to check the data confirmation of the new classification.	**Carcinogenicity and Mutagenicity** *In November 2005* the discussion was postponed to the next meeting due to the vast amount of information. IND would provide the TC C&L with summaries over the available information. This was circulated with the Follow-up sheet I in document ECBI/112/04 Add. 14. In preparation of the March 2006 meeting, IND sent in proposal for further testing ECBI/112/04 Add. 17. It was pointed out that all testing was performed on an ortorombic crystalline form that was not the product of commercial interest. *In March 2006* it was understood that new information from IND would most probable not be available in time for the October 2006 meeting. However the TC C&L agreed to postpone the discussion until the next meeting to give an additional possibility for all Member States to take their informed position to the classification. In preparation for the *October 2006 meeting* IND submitted ECBI/112/04 Add. 19 announcing which further studies are going to be conducted to fill data gaps. The new test program and its time schedule was described by IND and the TC C&L agreed at the meeting to await the results before continuation of the carcinogenicity and mutagenicity discussion. **IND** was asked to provide a paper in preparation for the next meeting or as soon as possible, summarising whether and how cross reading can be done between vanadium pentoxide and the other vanadium forms. They should confirm for which species of vanadium pentoxide different data (positive or negative) were provided and whether it would be considered applicable to vanadium pentoxide in all its forms. **IND** was also invited to provide the TC C&L with any preliminary results from the on-going studies as soon as available. **IND** sent document ECBI/112/04 Add. 21 in which it is explained that read across from vanadium pentoxide to other vanadium forms is not possible. Furthermore this document contains an update of the further inhalation studies performed.

	MS were asked to send their comments to ECBI/112/04 Add. 21 within the deadlines for the September meeting, and IND was asked to provide any additional information to be discussed at the meeting. **IND** sent further progress report on their on-going testing in ECBI/112/04 Add. 22 (I, II and III) distributed with Revision 5 of the September agenda. No additional comments from MS were received. ***In September 2007*** the TC C&L agreed to postpone the further discussion on the classification of vanadium pentoxide. They agreed to await additional data to be provided by IND when the current testing would be finalised. IND stated that they did not support any differentiation between classification of different species of vanadium pentoxide, why the earlier request from Member States on information on different species tested and marketed, would no longer be relevant. In addition the TC C&L suggested that in case they would end up classifying vanadium pentoxide as a carcinogenic category 2 it could be more relevant to apply R49 rather than R45, as probable other routes of exposure besides the inhalation route could be excluded. **⇨ Hand-over to ECHA**

2. FOLLOW-UP OF OTHER ISSUES THAN CLASSIFICATION OF SUBSTANCES

Outcome of the meeting of the ad-hoc Working Group on Aerosols (26th September 2007, back to back to the TC C&L meeting)

The first guidance document (ECBI/76/06), as written by Prof. Pauluhn was discussed at the 6 October 2006 ad-hoc meeting. Comments had been received by BE (ECBI/76/06 Add. 1) and NL (ECBI/76/06 Add.2). For the March 2007 TC C&L meeting, which was cancelled, Prof. Pauluhn provided a revised version of the guidance document (ECBI/76/06 Rev.1), taking into account the comments raised at the October 2006 meeting. Prof. Pauluhn presented the revised version at this meeting.: A re-structuring of the contents had been made, a new heading "Applicability of split-entry" had been introduced including new text and a figure to clarify the pre-requisites for the derogation from classification to be applied. A "Summary and Conclusions" also contained a justification of the principle. The "Background and Scope" had been revised and a number of examples had been introduced. The split entry approach could only be used under certain very specific circumstances e.g. when data were available excluding that death was caused by larger particles and showing particle sizes at the end-use (e.g dissolution and spraying). The title to the document will be changed to: "Modification of classification and labelling of acute inhalation toxicity" to better reflect the content.

One expert agreed to the principles although some would appreciate further clarifications. Experts were urged to send in written comments.

It has to be agreed by the RIP 3.6 health Working Group if this subject should be prioritised under RIP 3.6. In case it will not be prioritised, the work will be handed over to the European Chemicals Agency (ECHA).

After the meeting, ECB received a revised version from Prof. Pauluhn, including the NL and BE comments to ECBI/76/06, which were accidentally not included in the Revision 1 (see ECBI/76/06 Revision 2). Furthermore, it contains a new Appendix VII (including application of the GHS criteria) of the guidance document and considers comments provided at the meeting.

All TC C&L experts and experts from the Ad-hoc working group are invited to send further comments on the new revised version (ECBI/76/06 Rev. 2) during the follow up procedure.

After FU1:
Tom Gebel, DE has provided a revised version of the Rev. 2 document (ECBI/76/06 Rev. 2 Add. 1 parts I and II) in order to facilitate the understanding of the document on the basis of the comments and discussions at the last ad hoc WG meeting 26 Sept 2007.

NL has provided detailed comments to the Rev. 1 document in ECBI/76/06 Rev. 1, Add. 1

After FUII Professor Pauluhn has supplied ECBI/76/06 Rev. 3, dated 21st November 2007. To that version comments by DE (ECBI/76/06 Rev. 2, parts I and II are not yet included.

Another revision, ECBI/76/06 Rev. 4, was discussed at the RIP 3.6 working group 2 meeting in January 2008. It was agreed not to include this document as it is in the RIP 3.6 guidance document.

Outcome of the meeting of the ad-hoc Working Group on potency of reproductive toxic substances (Friday 28 Sept 2007, back to back to the TC C&L meeting)

After a teleconference that was held 20th June 2007, a revision of the document "The potency of substances inducing reproductive toxicity", was distributed by Andre Muller (ECBI/54/07 Part II). Also comments from the experts to the previous version and responses by Andre (ECBI/54/07 Part III), had been sent. Suggested items for discussion at this meeting were listed in ECBI/54/07 Part I. In addition, ECB suggested the group to finally agree on the definition of potency as well. (The current proposal reads: *"Reproductive toxicity potency is defined as the magnitude of reproductive toxic effects with respect to the dose of a chemical considering the study design in terms of species and strain, exposure route, exposure duration, exposure window in the life cycle, and possible concomitant parental toxicity")*

The experts agreed that for the potency determination of reprotoxic substances, both the dose and nature and severity of effects produced are important. However, the word "magnitude "in the current proposal, may not sufficiently explain this to everyone although it is an established concept within the area of carcinogenicity. *Thus, experts were asked to provide suggestions for an alternative wording of the definition ("magnitude") in order for Andre to revise it.*

Frank Sullivan had produced a scheme with an example of how reproductive toxic substances could be categorised with respect to their potency (mainly based on dose and severity of effect) and how Specific Concentration Limits (SCLs) could be ascribed to these categories. This approach is in line with the current guidance for setting SCLs for carcinogenic substances (as well as for the EU Expert recommendations for sensitisers). For example, developmental toxicity was suggested to be characterised in four main types such as fetal

death, malformations, physical development (body weights, variants etc) and neuro-behavioural development, and each of the four types of effects should be divided into e.g. low, medium or high potency etc. The proposal was well received by the group and will be refined by Frank Sullivan until next meeting.

Whatever proposal for setting SCLs on the basis of potency the group would finally agree upon and propose, a robust and scientifically well justified rationale had to be provided for the proposal. Thus, in order to provide this, it was indeed necessary to continue the work to build up the data base. It was necessary to finally agree at the meeting on the *parameters for fertility and developmental toxicity* to be included in the data base in order to start collecting data to be included. For *fertility* it was agreed to include the dose for irreversible effects (in the list of parameters on p. 34) if known and available. For *developmental toxicity*, the dose levels causing effects on neuro-behavioural development should be included, as well as information on mode of action (in the list of parameters on p. 32), if available.
Although the number of parameters to be included in the data base was considered to be high by now, it was agreed to include all of them in a first trial. In the subsequent analysis of this trial, parameters that not were found useful or workable could be deleted in order to provide a proposal for the definitive parameters, to be considered in the development of a method establishing SCLs.
Thus, as a next step, all experts would try to collect data for all the agreed parameters for two substances classified for developmental effects and/or fertility. If possible, a balance should be created between Cat. 2 and Cat. 3 classifications. While doing this, the time needed for the evaluation of a substance should be noted.

Members of the ad-hoc working group were asked to forward the chemical names and the classification (including which categories) of the 2 substances to ECB or directly to the group within two weeks i.e before 12th October.

Andre will update the tables with parameters for fertility and development, in accordance with the discussions at the meeting and revise the definition of potency (on the basis of incoming proposals).

The data on the listed parameters for the 2 chosen substances should be provided by 15th of January 2008, in order to be discussed at the next meeting.

Next meeting of the ad-hoc working group took place the 24th January 2008, back to back to the 2nd meeting in the RIP 3.6 working group on health effects, 21-23 January 2008.

3.1 Progress under Directive 67/548/EEC

Information concerning the 30th and the 31st ATP from DG ENV was forwarded by ECB.

30th ATP:

The 30th ATP version adopted by the Committee in February 2007 has been notified to the World Trade Organisation (WTO) in June in accordance with the TBT agreement under the WTO. Several comments, mainly for Borates and Nickel carbonate, have been received from Member Countries. The Commission is currently preparing reply to the comments. The issue will be discussed at the next TBT committee in November.

The Commission will take into account the reaction of the Member Countries when adopting the 30th ATP version. So, in any case, the 30th ATP cannot be adopted before November, it could rather take place in December 2007 or January 2008.

In addition to the WTO issue, language check of the testing methods is not yet finalised. MS will be asked to work on it.

The implementation period for member states for the 30th ATP (1 June 2009 at the latest) will not be modified.

31st ATP:

Member states had been asked to comment on the draft proposal for the 31st ATP before the 13 September. There were only a few comments and therefore DG ENV would like to organise the vote by using the written procedure that was added to the rule of procedure of the Committee during the last TPC meeting, without having a pre-TPC meeting. This written procedure could be launched in November/December 2007. In the written procedure, MS can request a TPC meeting.

However, there is one issue that needs to be solved before a written procedure can take place; how to present entries that are split due to different classifications depending on particle size. This issue has been discussed for the perborates and tolylfluanid. The perborates were concluded in March 2006 with "aerodynamic diameter of below 50 µm" as particle size cut-off in the entries. The issue was last discussed for tolylfluanid at the October meeting 2006 where the conclusion was to include the 50 µm diameter in the split entry, but in the FU III ECB concluded "Since UK supported DE to include 'thoracic fraction' in the entry for tolylfluanid, this will be proposed to be added to the agenda for the pre-TPC meeting of the 31st ATP." At the September meeting 2007, the TC C&L agreed to try to solve this in writing during Follow up.

In the FU document ECBI/60/07 tolylfluanid (613-116-007 and 613-116-01-4) and the perborates (005-017-00-7, 005-017-01-4, 005-018-00-2 and 005-018-01-X) are listed, with the particle size expressed as "aerodynamic diameter of below 50 µm" or "thoracic fraction according to EN481".

MS were asked to give their opinion on whether they prefer "aerodynamic diameter of below 50 µm" or "thoracic fraction according to EN481" in these entries before 7 November 2007.

-Belgium would prefer « aerodynamic diameter of below 50µm » rather than « thoracic fraction according to EN481 ».

-Netherlands prefer to use "aerodynamic diameter of below 50 µm" because this option is clearer and also more conservative until guidance is available describing how such a split entry has to be used in the determination of the classification of mixtures.

- Greece comments: Taking into account all the discussions held in ECB meetings, EL considers that for tolyfluanid split C&L entry the "aerodynamic diameter of below 50 µm" should be used.

-Sweden prefer to use "aerodynamic diameter of below 50 μm" in the entries in ATP31 of tolylfluanid and sodium perborates.

- Finlands position is that they would prefer "aerodynamic diameter of below 50 μm" since they believe that this provision is from a practical point of view easier to handle in an administrative environment.

ECB: On the basis of the responses from the five MS above, the "aerodynamic diameter of below 50 μm" will be included in the entries for tolylfluanid (613-116-007 and 613-116-01-4) and the perborates (005-017-00-7, 005-017-01-4, 005-018-00-2 and 005-018-01-X) in the proposal for the 31st ATP.

3.3 Hand over of files to the European Chemicals Agency (ECHA)

ECB reported that all concluded substances will not have to be handed over to ECHA by the Member States. These substances are candidates for inclusion into the lst ATP of the new CLP Regulation.
For non-concluded substances, MS will have to provide ECHA with a report in the Annex XV format and ECB will provide additional relevant documents related to the substance. If not provided, the discussion of the substance cannot continue. Document ECBI/57/07 contains the non-concluded substances, and the list has to be updated with the outcome of the 2007 TC C&L meetings. For all these substances, Annex XV report will have to be sent to ECHA, even if the Annex XV report was already submitted previously to ECB. New substances will also be included in this list.

After the meeting, ECB received some information about the planning of the first C&L discussions at ECHA.
The harmonised C&L of substances will be discussed by the Risk Assessment Committee (RAC). The Member States have been invited (at the end of September 2007) to nominate experts for the RAC.
The first RAC meetings are foreseen to take place in the beginning of 2008, where initially procedures and guidelines will have to be adopted.

4.1 Application of R64

Before the October 2006 TC C&L meeting, **UK** created a thought starter to develop a clearer framework for interpretation of the criteria for the application of R64, ("May cause harm to breast fed babies"). **DE** and **S** had commented and S sent their position from 1999. **UK** distributed a revised document shortly before the meeting. In an annex, **UK** listed which comments had been taken into account and which had not. The comments received highlighted technical issues that would need a detailed discussion, and should therefore be considered for RIP 3.6.

According to **UK**, the main issues for discussion were:
- Level of evidence needed for R64 classification. Do we need demonstrated evidence for an effect, or is just presence of a substance in the milk enough to cause concern.
- The possible influence of maternal toxicity on lactation, and secondary consequences.
- Are neonates always more sensitive than adults.

- Bioaccumulation in breast milk

It was agreed by the TC C&L that this issue should be forwarded to RIP 3.6 The Health Working Group under RIP 3.6 will consider which priority should be given to the subject.

ECB distributed the revised document from UK to the TC C&L with the first follow up sheet (See ECBI/117/06 Revision 1). TC C&L experts are welcomed to send any comments to ECB, which will be forwarded to the Health Working Group 2 under RIP 3.6.

After FUI:
DE: We think that one important point still has to be made clear in the revised document by the UK:
with respect to animal studies like e.g. multigeneration studies it can mostly not be proven that the adverse effect (the putative R64 effect) on the offspring was mediated via lactation or effects on lactation as the offspring was also prenatally exposed. Postnatal toxicity in the offspring can also be prenatally induced and thus it would be developmental toxicity. If this cannot be made clear, R64 cannot be assigned. The only general clear prove for R64 is a specific study design (cross-fostering).

NL agree with most changes in Revision 1 compared to the original document but have a number of comments as provided in document ECBI/117/06 Rev. 1 Add. 1. The document also includes a list of points of discussion based on the previous comments provided by SE and DE.

4.2 Compilation of GHS classification recommendations by the TC C&L

ECB created document ECBI/33/07 revision 1, with all the (concluded or not-concluded) substances with their GHS classification. MS experts were asked to confirm the GHS classification for the substances on the list.

Comments received have been included in Revision 2 of document ECBI/33/07. However, it still contains substances for which the GHS classification is not confirmed and updated in accordance with data.

Substances that are concluded, but for which the GHS classification is not confirmed cannot be included in the 1st ATP of the CLP Regulation. Therefore ECB will allow further commenting on the GHS classification of the substances in preparation for the 1st ATP. It should also be considered to organise a meeting for discussion of GHS classifications of concluded substances for the 1st ATP to the CLP Regulation.