The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Information Technology

EMEA/CVMP/511/01-FINAL

ANNEX I

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CVMP recommendations in preparation of Community comments on Codex Alimentarius MRLs for Veterinary Drugs

Comments on the Codex document CL 2000/28-RVDF: Request for comments at steps 6 and 3 on draft and proposed draft MRLs for veterinary drugs

Part 1. Draft MRLs at step 6

1. THIAMPHENICOL

1.1 Background

Comparison EU (CVMP)/draft CCRVDF(JECFA)MRLs:

	ADI	TARGET SPECIES	MARKER RESIDUE	MRLs (µg/kg)			
				Muscle	Fat	Liver	Kidney
EU (CVMP)	2.5 µg/kg bw	Porcine	Thiamphenicol	-	-	-	-
		Fish	"	-			
Draft CCRVDF (JECFA)	0-5 µg/kg bw	Porcine	Sum of thiamphenicol and thiamphenicol conjugates, measured as thiamphenicol	50	50	100	500
		Fish	"	50*			

* Muscle + skin

1.2 Consideration by the CVMP

Comments on the assessments of CVMP and JECFA

ADI:

The **CVMP** established a microbiological ADI of 2.5 µg/kg bw based on the mean MIC_{50} for *Fusobacterium* (0.50 µg/ml) and a bioavailable fraction of 0.5. This microbiological ADI, being lower than the toxicological one (45 µg/kg bw based on a NOEL of 9 mg/kg bw/day in a 13-week rat study and a safety factor of 200), was used for the calculation of MRLs.

The **JECFA** established a microbiological ADI of 4.58 µg/kg bw based on the mean MIC_{50} for *Fusobacterium* (0.50 µg/ml) and a bioavailable fraction of 0.4. In this case the JECFA considered that the NOEL of 5 mg/kg bw/day in the rat carcinogenicity study was the most relevant toxicological endpoint. Applying a safety factor of 100, the JECFA established a toxicological ADI of 0-50 µg/kg bw/day.

Although based on different endpoints, the toxicological ADIs set by the JECFA (0-50 µg/kg bw/day) and CVMP (45 µg/kg bw/day) are very similar.

MRLs:

The **JECFA** established temporary MRLs for fish and pig tissues. Due to lack of information on tissue metabolites, the marker residue was defined as the sum of thiamphenicol and thiamphenicol

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conjugates, measured as thiamphenicol. The JECFA noted that no data were available to determine the ratio of marker (MR) to total microbiologically active residues (TR) in any species. The JECFA recognised that thiamphenicol glucuronide is not microbiologically active, but could be converted in humans to microbiologically active parent drug after ingestion. Quantitative data on the presence of thiamphenicol glucuronide as a portion of the total residues were lacking. A validated analytical method for measuring the marker residue was not available. Furthermore, the JECFA felt that further work was needed to establish the distribution of metabolites in edible tissues.

In contrast to the JECFA, the **CVMP** identified the parent compound as marker residue. Similar to JECFA the CVMP considered the information on the ratio of marker residue to total microbiologically active residues inadequate and a lack of a validated analytical method

CVMP position:
In view of the deficiencies of the dossier assessed previously by the CVMP, the CVMP could not recommend the establishment of final MRLs for thiamphenicol for pigs and fin fish following the previous establishment of provisional MRLs in the EU, which expired on 1.1.2001. The CVMP noted very similar deficiencies in the dossier put forward by the JECFA.

In the present situation it is not possible to calculate MRLs, due to a severe lack of reliable data. It is, therefore, also not possible to check whether the MRLs established by JECFA result in a violation of the ADI of 2.5 µg/kg bw/day (= 150 µg/person/day)

Given the lack of information on the ratio of marker to total, it is not possible to support the marker residue defined by the JECFA (i.e. sum of thiamphenicol and thiamphenicol conjugates, measured as thiamphenicol). Likewise, there may even be insufficient ground for the marker residue initially proposed by the CVMP (i.e. the parent compound).

The CVMP recommended not supporting the draft CCRVDF MRLs.

Part 2. Proposed draft MRLs at step 3

2. CYHALOTHRIN

2.1 Background:

Comparison EU (CVMP)/draft CCRVDF (JECFA)MRLs:

	ADI	TARGET SPECIES	MARKER RESIDUE	MRLs (µg/kg)				
				Muscle	Fat	Liver	Kidney	Milk
EU (CVMP)	5 µg/kg bw	Bovine	Cyhalothrin (sum of isomers)	-	500	-	50	50
		Porcine		---	---	----	----	---
		Ovine		---	---	----	---	----
Draft CCRVDF (JECFA)	0-2 µg/kg bw*	Bovine	Cyhalothrin	20	400	20	20	30
		Porcine		20	400	20	20	N/A
		Ovine		20	400	20**	20	-

* Results of appropriate studies to establish a NOEL for neurobehavioural effects in laboratory animals are required for evaluation in 2002

** Results of the validation of the analytical method to demonstrate a limit of quantification of 0.01 mg/kg (sheep liver) are required for evaluation in 2002.

2.2 Consideration by the CVMP

Comments on the assessments of CVMP and JECFA

ADI:

The **CVMP** established a toxicological ADI of 5 µg/kg bw/day based on a NOEL of 0.5 mg/kg bw applying a safety factor of 100. The NOEL is based on a 52-week oral study in Beagle dogs, with administration of cyhalothrin in corn oil by gelatine capsules - neurological signs: muscular trembling, unsteadiness and vomiting. The safety factor was considered justified because the toxicity study was conducted with a suitable lipophilic vehicle.

The **JECFA** established an ADI of 2 µg/kg bw/day (120µg per person) based on a LOEL for induction of liquid faeces in a 26-week study in dogs. A safety factor of 500 was used, because of the absence of a NOEL for liquid faeces in dogs and because of the absence of a NOEL for neurobehavioral effects. The JECFA ADI is temporary and new studies to establish a NOEL for neurobehavioural effects in laboratory animals have been requested.

MRLs:

The **CVMP** identified cyhalothrin as marker residue in cattle. From the results of the different radiometric studies, it was estimated that cyhalothrin represents 100% of the total residues in muscle and fat, 20% in kidney and 90% in milk. In absence of radiolabelled studies in ovine and swine, no marker residue could be identified for the species. No method was available for monitoring residues of cyhalothrin in ovine milk and swine tissues.

No depletion studies using radiolabelled cyhalothrin were reported by the **JECFA** in any species. The JECFA claims to have suitable analytical methods validated for the edible tissues of the three species (bovine, ovine and swine), although the method in ovine liver has only a temporary status for validation. References for the validated analytical method in swine are scarce.

It is not possible to judge if the evaluation made by JECFA experts was based on the same studies that were available to CVMP.

The **JECFA** proposed for cyhalothrin temporary MRLs until 2002, until more toxicological results and a validated analytical method for ovine liver are presented. The same temporary MRLs are used for edible tissues from animal origin of cattle, pigs and sheep, and relating to the analytical method, it is stated that: (A) a suitable analytical method is available for analysis of cyhalothrin residues in edible tissues and milk. (B) MRLs for liver, kidney and muscle can be harmonised at twice the LOQ of the analytical method as validated for tissues from cattle and pigs. (C) MRLs for fat are based on the highest mean residues, plus 3 standard deviations, as determined in depletion studies using treatments consistent with good practice in the use of veterinary drugs. (D) The MRL recommended for milk is based on the highest mean residues, plus 3 standard deviations, as determined in depletion studies which used treatments with the spray formulation consistent with good practice in the use of veterinary drugs.

Based on the consumption of the international animal food basket, and with the marker to total residue ratio (liver: 1/16 and kidney 1/5, with the others as 1/1), the TMDI is 108 µg. The remainder of the ADI (12 µg) has been allocated to pesticide use.

Regarding the allocation of the ADI to residues from use as pesticide and veterinary drug, the **JECFA** proposed a TMDI of 108 µg (90%) for residues of animal origin and 12 µg (10%) for residues of vegetable origin.

CVMP position:
While the ovine MRLs can in principle be supported provided that a validated analytical method can be made available before the proposed MRLs would be advanced to step 7, the CVMP recommended not supporting the proposed draft CCRVDF MRLs in porcine species.

The reasons are that no radiolabelled depletion study is available for pigs, which does not allow to identify the marker residue in pigs, and the analytical method does not seem to be fully validated. Without data on these two points it is not possible to support approval of MRL values in pigs.

Furthermore, it should be noted that cyhalothrin MRLs are still under review in the EU and further comments may arise in the future.

3. DICYCLANIL

3.1. Background

Comparison EU (CVMP)/draft CCRVDF (JECFA)MRLs:

	ADI	TARGET SPECIES	MARKER RESIDUE	MRLs (µg/kg)			
				Muscle	Fat	Liver	Kidney
EU (CVMP)	7 µg/kg bw	Ovine	**Sum of dicyclanil and 2,4,6-triamino-pyrimidine-5-carbonitrile**	200	150	400	400
Draft CCRVDF (JECFA)	0-7 µg/kg bw	Ovine	**Dicyclanil**	200	150	400	400

3.2 Consideration by the CVMP

Comments on assessments of CVMP and JECFA

ADI:

JECFA and CVMP ADIs are identical and are based on the same data and approach. The ADI of 0.007 mg/kg bw (0.42 mg per person) was based on a NOEL of 0.7 mg/kg bw/day observed in a 12-month dietary toxicity study in dogs and a safety factor of 100.

MRLs:

Numerical figures for the MRLs are identical. However, the JECFA and CVMP approaches differ in the definition of the marker residue: CVMP MRLs refer to the sum of dicyclanil and the major metabolite 2,4,6-triamino-pyrimidine-5-carbonitrile while the JECFA has proposed the parent compound alone as the marker residue.

The tissue distribution of the two components of the CVMP marker residue may be described as follows: The metabolite 2,4,6-triamino-pyrimidine-5-carbonitrile was present in all tissues and represented the dominant residue fraction in liver and kidney, while parent compound predominated in fat. In muscle, both parent compound and 2,4,6-triamino-pyrimidine-5-carbonitrile were present in comparable amounts (see CVMP document EMEA/MRL/739/00-Final of May 2000).

Due to the difference in the marker residue between JECFA and CVMP, the ratios marker to total residues were also different: The ratio for parent dicyclanil to the total residues is lower (equal, at most) than that for the sum of parent dicyclanil plus the major metabolite. This difference in ratios was most noticeable in kidney, liver and muscle. Accordingly, a much larger correction factor was needed to estimate the theoretical maximum total residue intake (TMDI) resulting from JECFA MRLs. In consequence, JECFA MRLs, although numerically identical to CVMP MRLs, would lead to a higher residue intake.

The theoretical maximum daily intake (TMDI) calculated on the basis of the CVMP marker "sum of dicyclanil and 2,4,6-triamino-pyrimidine-5-carbonitrile" already represented about 100 % (> 98 %) of the ADI (see CVMP Summary Report EMEA/MRL/739/00-Final of May 2000). An estimate of the TMDI on the basis of the JECFA marker and its ratio to total residues showed that total residue intake would exceed the ADI by a factor of more than 3 (332.6 % of the ADI, see Annex 1).

CVMP position:

In conclusion, use of the JECFA marker "parent dicyclanil" would lead to an unacceptable exceedance of the ADI of 0.42 mg/person by the theoretical maximum daily residue intake.

Therefore the CVMP recommended not supporting the proposed draft CCRVDF MRLs.

Annex 1 (Dicyclanil)

Theoretical maximum daily intake of residues (TMDI)
(estimated on the basis of the marker residue "Sum of Dicyclanil plus 2,4,6-triamino-pyrimidine-5-carbonitrile" (CVMP) and for the marker "Dicyclanil" (JECFA)

Tissue	**Cf = consumption factor (kg)**	**MRL (µg/kg)**	**CVMP marker residue % ratio marker/total residues [1)]**	**JECFA marker residue % ratio marker/total residues [2)]**
Liver	0.1	400	15	4.5
Fat	0.05	150	100	92.3
Kidney	0.05	400	25	6.25
Muscle	0.3	200	100	33.3
			TMDI = 414.2 µg (98.6 % of ADI)	**TMDI = 1397.2 µg (332.6 % of ADI)**

$TMDI_{food\ basket} = MRL_{tissue} \times 100/(\%\ ratio\ marker/total)_{tissue} \times Cf_{tissue}$

[1)] Ratios for the sum of dicyclanil and 2,4,6-triamino-pyrimidine-5-carbonitrile as stated in the CVMP Summary Report EMEA/MRL/739/00-Final

[2)] Estimated from the ratios for the sum of dicyclanil and 2,4,6-triamino-pyrimidine-5-carbonitrile as stated in the CVMP Summary Report EMEA/MRL/739/00-Final and individual results for determinations of dicyclanil and of 2,4,6-triamino-pyrimidine-5-carbonitrile

4. IVERMECTIN

4.1 Background

Comparison EU (CVMP)/draft CCRVDF(JECFA) MRLs:

	ADI	TARGET SPECIES	MARKER RESIDUE	MRLs (µg/kg)				
				Muscle	Fat	Liver	Kidney	**Milk**
EU (CVMP)	1 µg/kg bw	Bovine	22,23-Dihydro-avermectin B1a	-	40	100	-	-
Draft CCRVDF (JECFA)	0-1 µg/kg bw	Bovine*	22,23-Dihydroaver-mectin B1a (H2B1a)	-	40✞	100✞	-	**10**

* Validation data on the analytical method and information on other routes of applications to cattle to evaluate the residues in milk are required for evaluation in 2002

✞ Tissue MRLs were already previously established by JECFA, under discussion is the milk MRL.

4.2 Consideration by the CVMP

The CVMP and the JECFA established the same ADI and MRLs for bovine tissues including values for fat and liver. Recently, the CVMP set MRLs for all edible tissues in deer leading to a maximum daily intake of 87% of the ADI. No MRL has been set in the EU for milk. Codex proposes now to set an MRL for milk at 10 µg/kg. Thus there is a concern that addition of an MRL for milk will result in a daily residue intake, which may exceed the ADI.

No information is available on the ratio of marker residue to total residues to calculate the ivermectin residue intake from milk. However, considering a ratio of 1 or 0.5 maximum daily residue intakes will amount 112% respective 137% of the ADI (see table).

	Daily residue intake [µg/person]	
Tissues	52	52
Milk (ratio 1)	15	
Milk (ratio 0.5)		30
Total	67	82
% of ADI	112	137

CVMP position:
The CVMP therefore recommended not supporting the proposed CCRVDF MRL.

5. TRICHLORFON (Metrifonate)

5.1 Background

The **JECFA** set an ADI of 0-20 µg/kg bw and recommended an MRL for bovine milk of 50 µg/l. MRLs were not recommended for muscle, liver, kidney or fat in cattle considering that no detectable residues should be present in tissues from animals treated with trichlorfon when used in accordance with good practice in the use of veterinary drugs. The limit of quantification may be used as a guideline maximum residue concentration in muscle, liver, kidney and fat (50 µg/kg).

The **CVMP** assessed trichlorfon (metrifonate) in 1999 but concluded that it was not possible to establish MRLs.

5.2 Consideration by the CVMP

Comments on the assessments of CVMP and JECFA
In its evaluation, the **JECFA** concluded that inhibition of acetylcholinesterase activity was the most relevant endpoint for establishing an ADI. The most appropriate NOEL was 0.2 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in humans treated orally. A safety factor of 10 was applied to this figure, giving an ADI of 0-20 µg/kg bw.

The CVMP could not establish an ADI due to the concerns regarding pharmacokinetics, teratogenicity, mutagenicity and neurotoxicity of the substance, and which are described below.

Pharmacokinetics:
Differences in pharmacokinetics between laboratory animals and humans comprise orders of magnitude. Generally, the pharmacokinetics of metrifonate in rodents and other laboratory animals appear to differ substantially from that observed in humans. Therefore, effects in humans may be expected at trichlorfon doses, which are lower by several orders of magnitude, than that inducing the corresponding effects in laboratory species.

Teratogenicity:
Trichlorfon is clearly fetotoxic and teratogenic in a number of laboratory species. Whereas NOELs for developmental toxicity could be identified in the mouse (~300 mg/kg bw, p.o.), rat (50 mg/kg bw, p.o.), hamster (200 mg/kg bw, p.o.), and rabbit (45 mg/kg bw, p.o.), severe teratogenic effects without NOELs were observed in guinea pigs and pigs.

In guinea pigs, oral doses of 100 mg/kg bw for 6 days at mid-gestation caused reduced brain weight with altered morphology and biochemistry resulting in locomotor disturbances in the offspring. No NOEL was established. In pigs, offspring of sows treated with daily doses of 40-100mg/kg bw for one to three days during mid gestation were observed to show congenital tremor with cerebral, predominantly cerebellar hypoplasia and loss of Purkinje cells. Again, a NOEL was not established. No explanation was given as to why JECFA dismissed the effects in these two species.

Mutagenicity:
The JECFA concluded that since the tests conducted *in vivo* produced mostly negative results when trichlorfon is administered orally, that the weight of evidence indicates that it is unlikely to represent a genotoxic risk. A ‘weight of evidence’ approach is often very subjective. In the case of trichlorfon, 20/33 in vitro studies and 4/9 in vivo investigations gave positive finding (see Annex 2). Trichlorfon is clearly genotoxic *in vitro*.

In addition to the 4 positive *in vivo* tests reported by the JECFA there are at least 3 other reports of induction of aneuploidy *in vivo* by trichlorfon that were not included in the JECFA assessment.

Czeizel (1994), reported highly statistically significant increases ($p<0.001$) in peripheral lymphocytes of 5 humans who attempted suicide by ingesting a trichlorfon-based pesticide. A similar highly significant increase was still present at 180 days in the 4 survivors.

Tian et al. (2000) reported high frequencies of micronuclei, mosaic aneuploidys and developmental retardation in embryos of pregnant female mice exposed to an acute i.p. dose of trichlorfon at 6 hr post presumed conception.

Sun et al. (2000) investigated spindle disturbances in vitro and effects on male germ cells *in vivo*. *In vitro*, trichlorfon (40-120 µg/ml) was a potent spindle poison in V79 cells. There was a dose-related increase in mitoses with spindle disturbances (>20-fold higher than controls at 120 µg/ml. In an *in vivo* FISH assay, single i.p. doses of 200-405 mg trichlorfon/kg bw caused a dose-dependent increase in disomic sperm.

Furthermore, dichlorvos to which trichlorfon is transformed, is mutagenic at the site of contact and no data are available to investigate this for trichlorfon. This is a particular cause for concern as trichlorfon is used topically and ingested residues in skin/fat/muscle are unlikely to have been extensively metabolised. The carcinogenicity studies on trichlorfon were also equivocal. There is also a convincing report of congenital effects in humans. A case-control study of a cluster of congenital abnormalities in a small Hungarian village found an association between ingestion of trichlorfon-contaminated fish and an increase of Down's syndrome cases, other malformations and twins. The Down's cases suggest a link between effects observed in laboratory species.

Neurotoxicity:
In the **CVMP** assessment, delayed neurotoxicity was observed in hens and primates ant toxic doses. Delayed neurotoxicity is regarded as a non-threshold effect by the CVMP. Again, thre is no information why JECFA dismissed these data.

ADI:
The **CVMP** were unable to set an ADI due to the concerns listed above. The **JECFA** set an ADI based on a NOEL from a prospective, double-blind, randomised clinical trail of trichlorfon in Alzheimer patients. Four groups of patients received daily oral doses at an initial loading dose of 0, 0.5, 0.9 or 2.0 mg/kg bw for two weeks in order to achieve steady-state cholinesterase inhibition quickly. This was followed by eight weeks of daily doses of 0, 0.2, 0.3 or 0.65 mg/kg bw daily. The intermediate and high doses improve cognitive function; the low dose had equivocal effects. Side effects were most severe in the high dose. The initial low dose of 0.5 mg/kg bw inhibited erythrocyte acetylcholinesterase by 29% and subsequent administration of 0.2 mg/kg bw maintained inhibition at 30-37%. The JECFA considered that as this dose enhanced inhibition by only 8% an insignificant change, 0.2 mg/kg bw was the NOEL for acetylcholinesterase inhibition.

There are major concerns regarding this ADI:

- The low dose was not a NOEL. Irrespective of the effects on cholinesterase, there were equivocal clinical effect (and side-effects) at this dose. At best it can be regarded as a LOEL, which required an additional uncertainty factor to determine an ADI.
- The derivation of a N(L)OEL of 0.2 mg trichlorfon/kg bw in human patients from a small increase of AChE inhibition after the reduction of 0.5 mg/kg bw loading dose to 0.2 mg/kg bw maintenance dose appears not based on sound scientific considerations.
- It is wholly inappropriate to base an ADI on a LOEL from clinical trail data involving a diseased sub/set of an aged sub-group of the human population.

CVMP position:
Considering the severe safety concerns outlined above the CVMP strongly recommended not supporting the establishment of MRLs for trichlorfon.

Annex 2 (Trichlorfon)

Summary of mutagenicity data (JECFA)

Study	Endpoint	Dose	Result	Reference
In vitro Interaction with DNA	7-methylguanine in mouse urine	160 mg/kg i.p.	**Positive**	Dedek *et al.* (1976)
	7-methylguanine in mouse liver and kidney	120 mg/kg i.p.	**Positive**	Dedek (1971)
Gene mutation (*rec*)	*B. subtilis* NIG17, 45	0.3 mg/disc-S9	Negative	Inukai & Iyatomi (1977)
	P. mirabilis PG273, 713	10 mg/spot-S9	**Positive**	Alder *et al.* (1976)
	B. subtilis H17, M45	NR-S9	Negative	Shirasu *et al.* (1976)
	B. subtilis H17, M45	2 mg/disc-S9	**Positive**	Shirasu *et al.* (1979)
	S. typhimurium	10 mg/disc	**Positive**	Jones *et al.* (1984)
Reverse mutation	*S .typhimurium* TA100, 98, 1535, 1538	5 mg/plate ± S9	**Positive** (TA 100 only)	Byeon *et al.* (1976)
	TA98, 100, 1535, 1537	0.5 mg/plate ± S9	Negative	Inukai & Iyatomi (1977)
	TA98, 100	~8.5 mg/plate ± S9	**Positive**	Batzinger & Bueding (1977)
	TA100, 1535	10 mg/plate ± S9	Negative	Zeiger *et al.* (1987)
	TA98, 100	2 mg/plate ± S9	Negative	Diril *et al.* (1990)
	TA1535, 1536, 1537, 1538 *E. coli* WP2/WP2*hcr*	NR-S9	Negative	Shirasu *et al.* (1976)
	TA1535, 1536, 1537, 1538	2 mg/disc -S9	Negative	Carere *et al.* (1978a/b)
	TA98, 100, 1535, 1537, 1538 *E. coli* WP2*hcr*	20 mg/plate ± S9	**Positive** (TA100, *E.coli*)	Shirasu *et al.* (1979) Moriya *et al.* (1983)
	TA97, 98, 100, 104, 1535	25 mg/plate ± S9	**Positive** (TA100, 104)	Barrueco *et al.* (1991)
	TA98, 100, 1535, 1537 *E. coli* WP2*uvrA*	5 mg/plate ± S9	**Positive** (except TA98, 1537)	Watabe (1997)
	S. cerevisiae 632/4, 632/1b, 814/18b	NR-S9	Negative	Guerzoni *et al.* (1976)
	S. cerevisiae S138, S211a	10 mg/ml ± S9	Negative	Hoorn (1983)
	S. cerevisiae D7	40 mg/ml ± S9	**Positive**	Jones *et al.* (1984)
Mitotic crossing over, gene conversion	*S. cerevisiae* D7	40 mg/ml ± S9	**Positive**	Jones *et al.* (1984)
Forward mutation	*S. coelicolor*	2 mg/disc -S9	**Positive**	Carere *et al.* (1978a/b)
	S. pombe SP-198	30 mg/ml ± S9	**Positive**	Gilot-Delhalle *et al.* (1983)
	V79 cells	200 mg/ml -S9	Negative	Aquilina *et al.* (1984)
	L5178Y cells	200 µg/ml -S9 600 µg/ml +S9	**Positive**	Witterland (1984) Jones *et al.* (1984)
DNA damage	*E. coli* pol $^{+/-}$	10 mg/plate ± S9	Negative	Herbold (1984)
	E. coli SOS	NR ± S9	Negative	Xu & Schurr (1990)
UDS	EUE cells	1000 mg/ml -S9	**Positive**	Aquilina *et al.* (1984)
	1º rat hepatocytes	50 µg/ml -S9	Negative	Myhr (1983)
SCE	V79 cells	80 µg/ml -S9 60 µg/ml +S9	**Positive**	Chen *et al.* (1981, 1982)
	CHO cells	100 µg/ml -S9 2 mg/ml -S9	**Positive**	Jones *et al.* (1984) Putman (1987)
Chromosomal damage	Don-6 cells	250 mg/ml -S9	**Positive**	Sasaki *et al.* (1980)
	Human lymphocytes	30 mg/ml -S9 3000 mg/ml +S9	**Positive**	Herbold (1986)

In vivo Reverse mutation	Host Mediated assay in mice TA98,100	200 mg/kg p.o.	**Positive** (TA100)	Batzinger & Bueding (1977)
Recessive lethal	*Drosophila*	4.5 mg/kg	Negative	Benes & Sram (1969) Brzheskiy (1973) Lamb (1977)
SCE	Chinese hamster bone marrow	300 mg/kg p.o.	Negative	Volkner (1987)
Chromosomal damage	Mouse bone marrow micronuclei	2 x 312 mg/kg i.p. 2 x 250 mg/kg p.o. 2 x 400 mg/kg p.o. 400 mg/kg p.o. 400 mg/kg p.o. (+) en 600 mg/kg p.o. (-) en	**Positive** (-)enantiomer only	Paik & Lee (1987) Herbold (1979a) Jones *et al.* (1984) Herbold (1997)
	Metaphase analysis in mouse bone marrow	400 mg/kg p.o. 10 mg/kg i.p.	**Positive**	Kurinnyi (1975) Moutschen-Dahmen *et al.* (1975) Degraeve *et al.* (1982, 1984) Nehes *et al.* (1982)
		100 mg/kg i.p. 0.5 mg/ml water (5dy/wk 7wks) 405 mg/kg i.p.	Negative	
	Metaphase analysis in hamster bone marrow	250 mg/kg i.p.	Negative	Dzwonkowska & Hubner (1986)
	Metaphase analysis in rat bone marrow	250 mg/kg p.o.	Negative	Bootman & Hobson-Walker (1987)
	Metaphase analysis in mouse spermatogonia/ Spermatocytes	1.5 mg/ml in water 50-100 days	**Positive**	Bulsiewicz *et al.* (1976) Moutschen-Dahmen *et al.* (1981) Degraeve *et al.* (1982, 1984) Herbold (1992)
		100 mg/kg bw i.p. 0.5 mg/ml water (5dy/wk 7 wks) 100 mg/kg i.p.	Negative	
	Dominant lethal mutation in mice	100 mg/kg i.p. 0.5 mg/ml water (5dy/wk 7 wks) 280 mg/kg i.p. 405 mg/kg i.p. 250 mg/kg p.o. NR	Negative	Estein *et al.* (1972) Dedek *et al.* (1975) Fischer *et al.* (1977) Herbold (1979b,c) Becker & Schoneich (1980) Moutschen-Dahmen *et al.* (1981) Degraeve *et al.* (1982, 1984) WHO (1992)
		405 mg/kg i.p. 405 mg/kg i.p. 54 mg/kg i.p. 3 weeks	**Positive**	