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ADVISORY COMMITTEE ON PESTICIDES

FOOD AND ENVIRONMENT PROTECTION ACT 1985, PART III

Control of Pesticides Regulations 1986

Evaluation of Fully Approved
or Provisionally Approved Products

Evaluation on: BOOSTER BIOCIDES IN ANTIFOULING PRODUCTS
FULL REVIEW OF DICHLOFLUANID

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NB: This document reflects the outcome of the 278th Advisory Committee on Pesticides meetings held in September 2000.

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LIST OF ABBREVIATIONS

Dimensions Symbols

^{0}C	degrees Celsius
cm	centimetre(s)
cm^{-2}	square centimetre(s)
µm	micrometre(s)
µg l^{-1}	microgramme(s) per litre
µl	microlitre(s)
µm	micrometre(s)
g	gramme(s)
g/m^3	gramme(s) per cubic metre
kg ai ha^{-1}	kilogrammes of active ingredient per hectare
kPa	kilopascals
Mbq	megabequerel(s)
mg	milligramme(s)
mg l^{-1}	milligramme(s) per litre
mg ai g^{-1}	milligramme(s) active ingredient per litre
mg ai l^{-1}	milligramme(s) per litre
mg C/kg	milligramme(s) of carbon per kilogramme
mg kg^{-1}	milligramme(s) per kilogramme
mg kg^{-1} bw	milligramme(s) per kilogramme of body weight
min	minute(s)
ml	millilitre(s)
ml l^{-1}	millilitres per litre
mm	millimetre(s)
mmol	millimoles
ng ai l^{-1}	nanogramme(s) active ingredient per litre
nm	nanometre(s)
Pa	pascal(s)
ppm	parts per million

A

ACP	U.K. Advisory Committee on Pesticides
ADME	adsorption, distribution, metabolism, elimination
AFP(s)	antifouling product(s)
ai	active ingredient
ALP	alkaline phosphatase
AR	applied radioactivity
AST	aspartate aminotransferase
ASTM	American Society for Testing and Materials

B

BBA	Biologische Bundesanstaff fur Land und Forstwirtschaft (Federal Biological Institute for Agriculture and Forestry - Germany)
BCF(s)	bioconcentration factor(s)
BSP	bromosulphopthalein
BUN	blood urea nitrogen
bw	body weight

C

^{14}C	radiolabelled carbon
$^{14}CH_4$	radiolabelled methane
CAS	Chemical Abstracts Services
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CEPE	European Confederation of Paint, Printers' Inks and Artists' Colours Manufacturers Association
CHO	chinese hamster ovary
CL	confidence limit(s)
CO_2	carbon dioxide
COD	chemical oxygen demand
COPR	The Control of Pesticides Regulations 1986 (U.K.)
D	
d	day(s)
DETR	U.K. Department of Environment, Transport and the Regions
DIT	DNA synthesis inhibition test
DMF	dimethylformamide
DMSA	N,N-dimethyl-N-phenylsulfamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
E	
EA	Environment Agency (U.K.)
EC_{50}	effective concentration (50%)
EC_{80}	effective concentration (80%)
EC_{90}	effective concentration (90%)
E_bC_{50}	effective concentration (50%) biomass
E_rC_{50}	effective concentration (50%) growth rate
EINECS	European Inventory of Existing Commercial Chemical Substances
EM	electron microscope
EPA	Environmental Protection Agency (U.S.A.)
EURATGD	European Risk Assessment Technical Guidance Document
F	
FADU	fluorescence assay of DNA unwinding
FID	flame ionisation detector/detection
FIFRA	Federal Insecticide, Fungicide and Rodenticide ACT (U.S.A.)
G	
gammaGT	gammaglutamyl transferase
GC	gas chromatography
GFR	glomerular filtration rate
GI	gastro-intestinal
GLP	good laboratory practice
GSH	glutathione
H	
h	hour(s)
HPLC	high performance liquid chromatograph(y)
HGPRT	hypoxanthine guanine phophoribosyl transferase
HSC	U.K. Health and Safety Commission
HSE	U.K. Health and Safety Executive
I	

IDS	U.K. Interdepartmental Secretariat
IMO	International Maritime Organisation
i.p.	intraperitoneal
IR	infra-red spectroscopy
ISO	International Standards Organisation
IUPAC	International Union of Pure and Applied Chemistry
i.v.	intravenous
K	
K_o	organic carbon absorption coefficient
K_1	uptake constant
K_2	depuration rate constant
L	
LC_{50}	median lethal concentration / lethal concentration (50 %)
LD_{50}	median lethal dose / lethal dose (50 %)
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
LOEL	lowest observed effect level
LSC	liquid scintillation counting
M	
MCHC	mean corpuscular haemaglobin concentration
MEC(s)	measured environmental concentration(s)
MEPC	Marine Environment Protection Committee
MIC	minimum inhibitory concentration(s)
MMAD	mass median aerodynamic diameter
MS	mass spectrometry
N	
NCE:PCE	normochromatic erythrocytes:polychromatic erythrocytes
NMR	nuclear magnetic resonance spectroscopy
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
O	
OECD	Organisation for Economic Co-operation and Development
P	
PAH	p-aminohippuric acid
PEC(s)	predicted environmental concentration(s)
$PEC_{sediment}$	predicted environmental concentration(s) in sediment
PEC_{water}	predicted environmental concentration(s) in water
PNEC	predicted no-effect concentration(s)
p.o.	per oral
Pow	octanol/water partition coefficient
PSD	U.K. Pesticides Safety Directorate
Q	
QA	quality assurance
QWASI	quantitative water / air / sediment interface
R	
REMA	Regulatory Environmental Modeling of Antifoulants
RH	relative humidity
rpm	revolution(s) per minute

S

s.c.	subcutaneous
SCE	sister chromatid exchange
SD	standard deviation
SPC	self polishing co-polymer
STP	sewage-treatment processes

T

T_0	test initiation time
$T^1/_2$	half-life / half-lives
TBT	tributyltin
TBTM	tributyltin methacrylate
TBTO	tributyltin oxide
TCC	thiazolidine-2-thione-4-carcboxylic acid
TID	thermonic detector
TLC	thin layer chromatograph(y)
TK	thymidine kinase

U

UDS	unscheduled DNA synthesis
UKAEA	United Kingdom Atomic Energy Authority
UV	ultra violet

V

v/v	volume for volume

W

w	week(s)
WHO	World Health Organisation
WP	wettable powder
w/v	weight for volume
w/w	weight for weight

OVERALL SUMMARY

Introduction

Dichlofluanid has been used as a fungicide in approved agricultural pesticides since 1965, in wood preservative products since 1978 and as a 'booster biocide' in antifouling products since before 1987. The antifouling products are approved for use on vessels of any size plus structures below the waterline. Dichlofluanid may also be used in aquaculture. The maximum concentration of dichlofluanid permitted as an active ingredient within approved antifouling formulations is 10.0 % w/w.

In 1995, the ACP considered reviews of triorganotin compounds (TOTs) and copper compounds in antifouling products. It concluded that the risk from the use of TOTs was unacceptable but that the use of copper antifouling products (AFPs) was acceptable. However the ACP recommended that a high priority should be placed on reviewing the organic booster biocides, since restrictions on the use of TOT-containing products could lead to an increased environmental loading of these compounds. 'Booster biocides' is a generic term given to a group of compounds normally found in addition to copper and occasionally TOT in AFPs. The purpose of these compounds is to enhance the products' efficacy against a broader spectrum of fouling organisms than that achieved with copper alone. 'Booster biocides' are either organic or organo-metallic compounds which have fungicidal, herbicidal or anti-microbial actions.

The ACP considered the full review of dichlofluanid at its 278th meeting in September 2000.

Recommendations

The ACP recommended that approvals for professional use of antifouling products, containing dichlofluanid at a maximum concentration of 10% w/w and to be applied by brush, roller, spreader or spray be continued, subject to conditions and data requirements which are set out in full in Section 9 of this document.

The ACP considered that the skin sensitisation potential of dichlofluanid was of sufficient concern to recommend that the amateur application of antifouling products by spray or aerosol be revoked. However it recommended that amateur application by brush and roller was acceptable with a recommendation for gloves to be worn as a precautionary measure. Full conditions and data requirements are also set out in full in Section 9 of this document. Data have now been submitted in response to these data requirements (November 2002).

Ministers accepted these recommendations.

Physical Chemistry

Dichlofluanid is the BSI name for N-dichlorofluoromethylthio-N', N'-dimethyl-N-phenylsulfamide (IUPAC) which has a purity of 98 - 99 % w/w. It is a colourless crystalline solid of molecular mass 333.2, melting point 105 ^{o}C, bulk density (loose) 400 kg m^{-3}, vapour pressure 3.79 x 10^{-5} Pa (at 25 ^{o}C); it is sparingly soluble in water (1.3 mg l^{-1} at 20 ^{o}C) and

has a log Pow 3.70 (at 21 ^{o}C). Acceptable analytical methods have been provided for the determination of dichlofluanid and impurities in technical material. An analytical method to determine dichlofluanid in water was provided although no limit of quantitation was given. Data requirements have been identified .

Mammalian Toxicokinetics

No human data were available to address the toxicokinetics of dichlofluanid. No data were presented regarding the toxicokinetics of dichlofluanid following inhalation or dermal administration.

Four oral dosing toxicokinetic studies were available, all performed in the rat. The first investigated the ADME of ^{14}C ring labelled dichlofluanid. The second investigated the faecal metabolites of ^{14}C ring labelled dichlofluanid. A further two studies investigated the generation and fate of the fluorodichloromethyl sulphenyl group.

Following oral dosing, 80-90 % of the administered radiolabel was absorbed. Following absorption the radiolabel was widely distributed, with the principle locations being thyroid and liver. No data were presented which indicated that dichlofluanid would bioaccumulate. Dichlofluanid is initially metabolised, via non-enzymatic reactions with cellular thiols, such as cystein or glutathione (GSH), to yield N,N-dimethyl-N-phenylsulphamide (DMSA) and the fluorodichloromethyl sulphenyl moiety. The DMSA undergoes further hydroxylation and N-demethylation, and phase II conjugation reactions. The fluorodichloromethyl sulphenyl conjugate is further metabolised via reaction with GSH or cystein to eventually form thiazolidine-2-thione-4-carboxcylic acid (TCC). The amount of TCC generated was apparently independent of route of administration, but less TCC was generated following high dose administration at the low dose. This could be as a result of a secondary, low affinity high capacity pathway becoming involved. The principle route of elimination was via the urine for all metabolites. Following administration of dichlofluanid radiolabelled at fluorodichloromethyl sulphenyl group, a shift from urinary to faecal elimination was noted at the top dose, a possible indication of depletion of cellular GSH pools. At 5 mg kg^{-1}, 22 % of the radiolabel was detected in the expired air.

No evidence of toxicity was observed following acute dermal application, suggesting dichlofluanid does not cross the skin. It is apparent that dichlofluanid is systemically available following oral dosing. No acute or repeated dose toxicity information is available via the inhalation route of exposure, therefore no predictions can be made as to the toxicokinetics via this route.

The available data indicate that dichlofluanid is extensively and rapidly metabolised and these metabolites are eliminated via the urine, suggesting both a low molecular weight and high water solubility. It is likely that the toxicokinetics of dichlofluanid observed in the rat will be similar in humans. No further data requirements have been identified.

Mammalian Toxicology

Acute Toxicity *- An acute oral NOEL was established in the rat, of > 1000 mg kg^{-1} , with an LD_{50} of > 5000 mg kg^{-1}. The female guinea pig and female rabbit were more sensitive, and mice slightly less sensitive, than the rat to an acute oral dose of dichlofluanid. The dermal LD_{50} was > 5000 mg kg^{-1} in the rat. Inhalation exposure in the rat (1 h, head only) found the LC_{50} to be > 2469 mg m^{-3} and the NOEC < 2469 mg m^{-3}. A four-hour exposure (head only), carried out in the rat found the LC_{50} to be 1338 and 1233 mg m^{-3} respectively. A NOEC was not determined as deaths were reported at all doses. Inhalation exposure in the rat was found to be the most sensitive acute toxicity end-point. Dichlofluanid is classified as harmful by inhalation.*

Dichlofluanid was not classified as a skin or eye irritant. Three sensitisation studies were carried out : a Magnusson and Kligman guinea pig maximisation test; a Draize sensitisation study; and a Klecaks open epicutaneous test. The Magnusson and Kligman test was positive. Both the Draize sensitisation study and Klecaks open epicutaneous test were considered by the authors to be positive. On the basis of these three studies it was concluded that dichlofluanid should be classified as a sensitiser.

The eye and skin irritation potential of two organic solvent and one spirit-based dichlofluanid-containing formulations (containing 0.4 % dichlofluanid of 88 % purity) were assessed. None of these formulations was classified as an irritant. An acute oral toxicity study found the NOEL of an organic solvent-based dichlofluanid-containing formulation (containing 0.4 % dichlofluanid of 88 % purity) to be 6.2 ml kg^{-1}; equivalent to 66 mg kg^{-1}. The acute dermal NOAELs of two spirit-based formulations containing dichlofluanid (containing 0.4 % or 1 % dichlofluanid of 88 % purity) were 2.5 ml kg^{-1}; equivalent to 14 and 22 mg kg^{-1} respectively. These data indicated the formulations to be more toxic than the active ingredient, by both the oral and dermal route.

Repeat-Dose Toxicity *- Dichlofluanid has been tested for repeat-dose toxicity in the rat, in a 4-month rat dietary and two lifetime studies, in two mouse lifetime studies, in 4-month and 2-year dog dietary studies and two 1-year capsule studies. No studies were presented with inhalation or dermal administration*

In a 1-year dog study clinical chemistry findings with supportive histopathology, indicative of nephrotoxicity and liver damage, were observed. In this study a NOAEL of 2.5 mg kg^{-1} d^{-1} was established.

The most consistent findings were cranial osteosclerosis in the rat, and findings consistent with fluorosis in a 2-year mouse study. A detailed assessment of cranial osteosclerosis was performed in a rat 2-year study, in which a LOEL was established at 10-14 mg kg^{-1} d^{-1}. A clear increase in the incidence of cranial osteosclerosis was observed in the low- and middle-dose groups, with almost all animals affected at the top dose. These findings are likely to be secondary to fluorosis in these animals. In terms of interspecies comparisons, in a mouse 2-year study, thickening of both the appositional bone of the cranial vault and nasal turbinates, and tooth alveolitis were observed at the top dose of 1,731-1,873 mg kg^{-1} d^{-1}. These findings are also considered to be a secondary consequence of fluorosis in these animals. No evidence of fluorosis was observed in the dog studies.

The findings are likely to represent a fluoride mediated perturbation of bone metabolism but are not considered to be of concern for human health. It is considered that the observed

renal and liver damage is of concern to human health. Data requirements have been identified.

Genotoxicity *- Dichlofluanid has been thoroughly tested for genotoxic potential in a range of in vitro and in vivo studies. Dichlofluanid was found to be a bacterial point mutagen, and cause mutations at the TK locus in eukayotic cells. A positive result was obtained in an in vitro cytogenetics assay. However, when tested in vivo, negative results were obtained in a mouse micronucleus, liver UDS and in one somatic cell chromosome aberration test. Further in vivo testing in a mouse spot test, a sperm cell chromosomal aberration cell test and a rodent dominant lethal test were all clearly negative. The available data indicate that dichlofluanid was not an in vivo somatic cell or germ cell mutagen. Overall dichlofluanid is unlikely to pose a genotoxic hazard to man.*

Chronic Toxicity *- The summary of the chronic toxicity has been combined with the summary of the repeated-dose toxicity. See Repeated-Dose Toxicity above.*

Combined Chronic Toxicity And Carcinogenicity *- The summary of the chronic toxicity of this chronic/carcinogenicity study has been combined with the summary of the repeated-dose toxicity. See Repeated-Dose Toxicity above.*

Carcinogenicity *- The only carcinogenicity data relate to the oral route of exposure, with studies performed in the rat and mouse. No evidence of carcinogenic potential was observed in the mouse. However, in the rat dichlofluanid was found to cause an increase in the incidence of thyroid follicular cell tumours at the highest dose level of 300-420 mg kg^{-1} d^{-1}. These tumours occurred at a single site, were of late onset and generally benign pathology. Dichlofluanid has been thoroughly examined for genotoxic potential and the available data indicate that dichlofluanid was not an in vivo somatic cell or germ cell mutagen. The results of the histopathological examination of the thyroid follicular cell tumours are consistent with a non-genotoxic aetiology. Overall, it can be concluded that dichlofluanid is a non-genotoxic rat thyroid follicular cell carcinogen.*

Rat follicular cell thyroid tumours can arise as a secondary consequence of perturbations in thyroid hormone homeostasis which produce prolonged stimulation of the thyroid gland by thyroid stimulating hormone (TSH) via a positive feedback mechanism. The data obtained from the specialised studies are consistent with a mechanism involving inhibition of thyroid peroxidase by the rat dichlofluanid metabolite thiazolidine-2- thione-4-carboxylic acid. Such inhibition will perturb thyroid hormone homeostasis leading to a prolonged TSH-mediated stimulation of the thyroid gland and eventually to tumour formation. The available in vivo data for dichlofluanid indicate that repeated dietary administration for 9 weeks in rats caused a decrease in T3 and T4 levels. Interspecies comparisons of the relative sensitivities of thyroid hormone homeostasis to disturbance by xenobiotics have shown that humans are markedly less sensitive than rats to such disturbances. Overall, it is considered unlikely that the rat thyroid tumours observed following repeated administration of dichlofluanid are of relevance to human health.

Reproductive Toxicity *- No effects on fertility, gestation or development were found in the three studies. In the 1992 study no treatment-related effects were found in parental animals. Reductions in pup body weight were reported at 900 ppm from both generations. The NOELs for the F0 parental animals was 900 ppm; equivalent to 72 and 79.4 mg kg^{-1} d^{-1} for males and females respectively. The NOEL for the F1 generation parental animals was 900 ppm;*

equivalent to 102.3 and 117.5 mg kg^{-1} d^{-1} for males and females respectively. The NOEL for pups was 90 ppm for both generations.
The 1991 two-generation study was carried out at higher dose levels. The major adverse effects were deaths throughout the study period and effects on skull growth. Where NOELs were identified they were lower than those in the 1992 study. In a poorly reported three-generation study NOELs were identified, although higher than those in the 1992 study.

Developmental Studies *- In three developmental toxicity studies (one in the rabbit and two in the rat), one of which was conducted to GLP, dichlofluanid was not found to be a developmental toxicant. In the rat, the NOEL for developmental toxicity was 500 mg kg d^{-1} with a maternal NOEL of 250 mg kg^{-1} d^{-1} based on decreased maternal body weight. In the rabbit, the NOELs were 30 mg kg^{-1} for maternal toxicity, embryotoxicity and fetotoxicity, and >100 mg kg^{-1} for developmental toxicity.*

Specialised Studies *- Two mechanistic studies are available, the first an in vitro study using preparations of TPO and Type 1 5'monodeiodinase enzymes and the second an in vivo repeated dose dietary study conducted in the rat. In the in vitro study, TCC showed the potential to reversibly inhibit TPO-catalysed iodine formation and TPO-catalysed tyrosine monoiodination. In the 9-week in vivo study, significant decreases in plasma T3 and T4 levels were noted on days 7 and 21 of the study at the highest dose level of 355 mg kg^{-1} d^{-1}. On day 63 plasma T3 and T4 levels remained decreased although not achieving statistical significance. A dose-dependent increase in thyroid weight was observed on day 7 of the study only. Data requirements were identified.*

No further data requirements have been identified.

Operator And 'Consumer' Exposure And Risk Assessments

Data generated by the manufacturers and by HSE estimate similar levels of exposure of professional workers during spraying. In the risk assessments for professional and amateur use, the NOAEL of 2.5 mg kg^{-1} d^{-1}, based on renal effects reported in the 1-year feeding study in the dog, has been employed. . Estimates of inhalation exposure use the LC_{50} value of 1233-1388 mg m^{-3}.

The risk assessment for professional users for application by spray, brush, roller and spreader and aerosol gives some cause for concern. However the risk assessment was conservative, with exposure calculations based on an estimated 10 % skin penetration of dichlofluanid. A dermal penetration study is requested to refine the assessment of exposure. The ACP considered that the skin sensitisation potential of dichlofluanid was of sufficient concern to recommend that the amateur application of antifouling products by spray or aerosol be revoked. However it recommended that amateur application by brush and roller was acceptable with a recommendation for gloves to be worn as a precautionary measure.
No data are available for bystander exposure to antifouling products. However, the risk of skin sensitisation for bystanders is considered minimal.

Further data requirements have been identified.

Environmental Fate And Behaviour *- Dichlofluanid was observed to hydrolyse instantly at pH 9, so rapidly that no parent compound could be detected. At pH 7 and 20 ^{o}C, a half-life of*

25.6 h was calculated. Further hydrolysis studies with the primary metabolite, dimethylaminosulfanilide, indicated that half-lives were in excess of 1 year at all pHs tested.

The degradation of dichlofluanid in soil was investigated in a number of studies. Aerobic and anaerobic studies resulted in degradation of dichlofluanid to the primary metabolite dimethylaminosulfanilide and in anaerobic studies, degradation to a further metabolite methylaminosulfanilide was noted. Half-lives of 2-5 d were calculated for aerobic conditions. Anaerobic half-lives were not calculated.

Several mobility studies confirmed that dichlofluanid was immobile. However, the primary metabolite dimethylaminosulfanilide was classified as immobile to slightly mobile (based on the Helling and Turner classification system).

Bioaccumulation studies on the bluegill sunfish indicated that dichlofluanid accumulated very rapidly (a steady state was reached at approximately 19 h) with a total residue bioconcentration factor of 73 for whole fish. However, residues were depurated quickly with a depuration half-life calculated as < 6 h. It can be assumed from this data that dichlofluanid is unlikely to bioconcentrate in fish.

Further data requirements have been identified.

Ecotoxicology *- The 96 h E_bC_{50} and E_rC_{50} values of the freshwater algae Scenedesmus subspicatus exposed to dichlofluanid were not established. The NOEC was quoted as the highest concentration tested, 1 mg l^{-1}. The 48 h EC_{50} for Daphnia magna was reported to be 0.42 mg l^{-1} with a NOEC of 0.07 mg l^{-1}. In a chronic Daphnia study, reproduction was significantly inhibited (51.7 %) at 0.2 mg l^{-1} . Dichlofluanid was acutely toxic to freshwater fish resulting in 96 h LC_{50} values of 0.01 and 0.03 mg l^{-1} for rainbow trout and bluegill sunfish respectively. NOECs were reported to be < 0.024 and < 0.0026 mg l^{-1} respectively.*

Dichlofluanid was found to be of low toxicity to birds with LD_{50} values in excess of 2226 mg kg^{-1} bw for acute oral studies and > 5 000 ppm for acute dietary tests.

No further data requirements have been identified.

Environmental Risk Assessment

The risk assessment has been concentrated on the marine environment since the data available are predominantly for the use of antifouling products (AFPs) in estuarine and coastal areas; although, the risk to freshwater environments has not been precluded. However, the strategy for assessing risk to the marine environment is less well developed than for terrestrial or freshwater environments. Therefore, the risk assessment strategy adopted for the current review has been presented in a supporting document 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002). This document presents a comprehensive and comparative risk assessment for all approved booster biocides and has been endorsed by an expert ad-hoc Environmental Panel. Below are the main points of the risk assessment concerningthe use of AFPs containing dichlofluanid; however, reference to the complete document is advised.

Environmental Concentrations *- Usage information was taken from a survey conducted by the Environment Agency (EA) in 1998. The EA survey demonstrated that dichlofluanid was currently used on 2.1-3.2 % of pleasure-crafts in the U.K.. Seawater monitoring data provided by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in 1998 demonstrated that dichlofluanid was not detected at any of the sites. No sediment analysis was conducted for dichlofluanid. However, the current monitoring data could only ever represent the usage levels for 1998, and predictions of maximum PECs were considered necessary since post approval usage cannot be controlled. Therefore, PEC data based on 100 % usage (all vessels treated) of dichlofluanid AFPs were calculated using a model developed as part of an HSE/EA commissioned research project. The model, Regulatory Environmental Modeling of Antifoulants (REMA), is a steady-state quantitative water air sediment interface (QWASI) model designed to predict concentrations of biocides in both the water and sediment compartments of estuaries/marinas/harbours. From the calculations, concentrations of dichlofluanid in water were greater than the limit of detection quoted by CEFAS even at the low usage levels in both marinas and estuary scenarios. The mean PEC calculations for the sediment compartment were very low and would be difficult to detect as no analytical limit of detection for dichlofluanid in sediment has been derived.*

Predicted No Effect Concentrations *- Once released into the aquatic compartment, the chemical fate of the booster biocide will determine whether the toxic effect exerted is limited to the target organisms within a boundary layer of a painted surface, or whether the active ingredient persists and there is potential for exposure to non-target organisms. Therefore selection of key non-target organisms and likely duration of exposure is essential, but this is somewhat reliant on the availability of acceptable data for representative marine species. Chronic data end-points have been selected as more appropriate for the purpose of a marine risk assessment following the use of booster biocides. This is because the inputs of booster biocides into the marine environment as a result of leaching from multiple-point sources (treated surfaces) will be a continuous process. Comparisons between marine and freshwater chronic toxicity data for booster biocides has not demonstrated any differences in sensitivities. Therefore, freshwater data have been accepted. Further to this, the most sensitive species has been selected, regardless of test medium. The provision of safety factors were in accordance with the guidance detailed in the European Risk Assessment Technical Guidance Document [EURATGD, 1996], and those previously accepted by the ACP. Only freshwater chronic data were available for dichlofluanid. These included data on algae and Daphnia magna; the latter being the most sensitive species for which a 24 d NOEC (reproduction) of 40 μg ai l^{-1} was reported. Therefore, the dichlofluanid toxicity data attracted a safety factor of 50. The PNEC for dichlofluanid for the purposes of this risk assessment was 0.8 μg ai l^{-1} for Daphnia magna.*

Risk Quotient *- The PEC:PNEC calculations, derived from the predicted data based on 100 % usage for dichlofluanid, suggest that dichlofluanid would be of low concern with only 14.3 % of open marina sites reaching unacceptable levels. This suggests that the use of dichlofluanid in AFPs would not result in gross unacceptable effects. However, refinement of the safety factor with additional toxicity studies were considered necessary to address the risk from use on pleasure-crafts in U.K. open marinas. In addition, clarification of degradation and metabolite formation in aquatic systems have been requested. The $PEC_{sediment}$ data for dichlofluanid derived by the REMA model demonstrated that the sediment compartment was of low concern. Therefore, no additional data requirements have been requested to address fate, behaviour or toxicity in sediment.*

Efficacy

Limited efficacy test data generated using raft tests have been provided. These raft tests were performed at test sites at two different geographical locations. The fouling challenge is likely to have been different at the two sites, although details have not been provided for one of them and only limited information is available on the other.

Data were provided by only one of the companies who are Approval Holders for products containing dichlofluanid. These data were generated from studies conducted on a limited range of products representative of current approvals in respect of the levels of the "booster" dichlofluanid (present in combination with copper derivatives as the principal biocide).

All but one of the studies involved the testing of conventional products, the exception being a study conducted using a TBT-free ablative coating. All of the formulations tested contained copper as the principal active ingredient (as Cu_2O or CuSCN) and dichlofluanid. No other combinations of active ingredients were represented.

The results from the single raft test conducted on the TBT-free ablative coating product demonstrated that a satisfactory level of antifouling performance could be achieved.

There were few data points in the studies conducted on conventional products. However, the results provide some limited evidence of the efficacy of an antifouling formulation containing dichlofluanid.

No data have been specifically provided in support of products formulated using contact leaching technologies.

In view of the limited TBT-free ablative products, the paucity of data points for conventional products, and the absence of data for contact leaching products, it is recommended that approvals continue pending submission of further efficacy data on all product types.

1. INTRODUCTION.

1.1 INTRODUCTION AND BACKGROUND TO THE REVIEW

Dichlofluanid was first approved for use as a fungicide on strawberries in 1965, gained wood preservative use in 1978 and has been used in antifouling products since before 1987.

1.1.1 REVIEW OF ANTIFOULING USE

Following reviews of the use of triorganotins and copper compounds in antifouling products, Ministers agreed in 1995 that reviews of all the 'booster biocides', the organic active ingredients used in association with copper and/or triorganotins, should receive high priority. The ACP agreed a timetable to complete the hazard evaluations of these substances by 2000, so all of the reviews could be considered together and a concerted approach to the environmental risk assessment undertaken. The review of dichlofluanid was considered by the ACP in September 2000, at its 278th meeting.

1.1 REGISTRATION HISTORY OF ANTIFOULING PRODUCTS

1.2.1 BACKGROUND

In July 1987 antifouling products were brought under The Control of Pesticides Regulations (COPR) 1986. As a result of discussions between Government Departments and the antifouling product industry, it was agreed that these products would not be subject to the same registration procedures as other non-agricultural pesticides until their active ingredients were fully reviewed. Companies producing antifouling products had been invited in April 1987 to inform the HSE Pesticides Registration Section of all active ingredients used in the formulations that they wished to be included under the Regulations. Although many of these active ingredients had not been previously evaluated by the ACP, there was no requirement at that time to submit any safety or other data to support their Applications.

In addition, antifouling companies were permitted to seek approval for products where, unlike other pesticide products, the active ingredient(s) was (were) specified in terms of a percentage range instead of a fixed value; there were no restrictions placed upon the number of active ingredients permitted in each antifouling product.

It had been further agreed, and it was published in The Edinburgh Gazette of Friday 26 June 1987 to this effect, that antifouling products should be classified and labelled in accordance with the 77/728/EEC Council Directive relating to classification, packaging and labelling of paints, varnishes and inks (European legislation which has subsequently been superseded by the 88/379/EC Council Directive relating to the classification, packaging and labelling of dangerous preparations). As an outcome of this decision with respect to labelling of antifouling products, it was only necessary for the names of certain "dangerous substances" to appear on product labels rather than each active ingredient and its percentage in w/w.

The first approvals of antifouling products containing dichlofluanid, granted on 29 June 1987, were given under these transitional arrangements.

In 1991, proposals attempting to terminate the transitional arrangements granted to antifouling products were presented to the ACP so that all pesticide products would be dealt in a similar fashion. However, it was considered prudent to leave these arrangements in place until a full review of all active ingredients found in antifouling products had taken place.

1.2.2 PROPOSED INTERNATIONAL BAN ON ORGANOTINS

On 22 November 1999 the IMO General Assembly passed a resolution, put forward by the Marine Environment Protection Committee (MEPC), that that the MEPC should develop a global, legally binding, instrument to address the harmful effects of antifouling systems used on ships. The Assembly also agreed that the instrument should ensure a global prohibition of the application of organotin compounds by 1 January 2003 and the prohibition of its presence on ships by 2008.

A working group within MEPC has started to draft a free-standing international convention to regulate shipboard antifouling systems that have adverse effects on the marine environment. This will include an annex of systems subject to specific controls, including a ban on their use. The IMO Council agreed that there should be a Diplomatic Conference on the convention in 2001.

It should be noted that any ban on the use of organotins in antifouling products could result in increased use of any or all of the booster biocides.

1.2.3 CURRENT ANTIFOULING PRODUCTS CONTAINING DICHLOFLUANID

As of November 1999, there were 270 approved antifouling products that may be marketed legally within the U.K.. Of these, 23 (approximately 8.5 %) actually contain dichlofluanid as one of their active ingredients.

Dichlofluanid is an active ingredient used in approved professional and amateur use antifouling products for use on vessels of any size plus structures below the waterline such as oil platforms, jetties, navigation buoys or piers. Dichlofluanid may also be used in aquaculture (on apparatus or equipment used in the cultivation of fish and shellfish plus fishing nets, lobster pots etc.). The maximum concentrations of dichlofluanid permitted as an active ingredient within approved formulations are :

products applied to vessels by amateur users	:	10.0 % w/w
products applied to vessels by professional users	:	10.0 % w/w

At present (November 1999), of the 23 currently approved antifouling products containing dichlofluanid, 22 products are for amateur and professional use; and 1 product is solely for professional use.

The distribution of dichlofluanid antifouling products by active ingredient is shown in Table 1.1.

Table 1.1 : Distribution Of Dichlofluanid Antifouling Products By Active Ingredient

active ingredient(s)	no.	active ingredient(s)	no.
dichlofluanid/Cu_2O	9	dichlofluanid/Cu_2O/diuron	1
dichlofluanid/CuSCN	5	dichlofluanid/Cu_2O/diuron/Irgarol 1051	1
dichlofluanid/Cu_2O/Irgarol 1051	1	dichlofluanid/diuron/zinc pyrithione	1
dichlofluanid/CuSCN/Irgarol 1051	1	dichlofluanid	1
dichlofluanid/CuSCN/Irgarol 1051/ diuron	2	dichlofluanid/CuSCN/Irgarol 1051/ zinc pyrithione	1

Application rates recommended by companies for products containing dichlofluanid vary depending upon expected service life of the antifouling coating. For example, for products applied at a rate of 1 litre per 4 m^2, surfaces will require repainting every 24-36 months whilst for those applied at a rate of 1 litre per 12 m^2, surfaces will require repainting every 6 months.

Standard application methods for applying approved products containing dichlofluanid to vessels/structures are by brush, roller, airless or conventional spray and spreader. In addition, provisional approval (amateur and professional use) has been granted for an aerosol product containing up to 1.5 % w/w dichlofluanid for use on outboard motors, rudders and propellers of small boats, with 1 litre of product typically covering 7.5 m^2 of surface.

1.2.4 CLASSIFICATION AND LABELLING OF ANTIFOULING PRODUCTS CONTAINING UP TO 10 % w/w OF DICHLOFLUANID

1.2.4.1 Background

Under transitional agreements reached between Government Ministers and Industry in 1987, antifouling products are classified and labelled as if they were paints and must comply with current EC legislation, namely Council Directive 88/379/EC relating to the classification, packaging and labelling of dangerous preparations; which is currently implemented in the U.K. as The Chemicals (Hazard Information and Packaging for Supply) Regulations 1994.

This “Dangerous Preparations” Directive (and its subsequent adaptations) allows EC classifications assigned to each substance within a preparation to be pooled together in order to determine overall EC Classifications for the product, by use of an agreed set of calculations and assumptions as laid out in an annex to this Directive.

The EC classification(s) for each substance used in this determination of product classification must be taken, whenever possible, from a European inventory - Annex I to Council Directive 67/548/EC relating to the classification, packaging and labelling of dangerous substances. This Annex contains EC classifications for a number of

compounds/elements which have been agreed by EC Member States after consideration of all available toxiciological and environmental data. Therefore, where substances have already been considered by EC Member States, decisions taken on their classification(s) will override any such decisions taken at a national level.

If a substance does not have an entry on Annex I of Directive 67/548/EEC, then data provided on the manufacturer's Material Safety Data Sheet for that substance should be used in determining overall classification.

However it should be pointed out that should actual formulation data be available, then this will override any determination of product classification which has been derived from the classifications of its component substances.

1.2.4.2 Classification / Labelling Under U.K. Legislation

Dichlofluanid itself has an entry on Annex I to Directive 67/548/EEC (reproduced in the U.K. as various editions of The HSC Approved Supply List) stating that it is classified as :

SENSITISING (Xi) : R43 : MAY CAUSE SENSITISATION BY SKIN CONTACT.
IRRITANT (Xi) : R36 : IRRITATING TO EYES.
HARMFUL (Xn) : R20 : HARMFUL BY INHALATION

N : DANGEROUS FOR THE ENVIRONMENT
R50 : VERY TOXIC TO AQUATIC ORGANISMS
R53 :MAY CAUSE LONG TERM ADVERSE EFFECTS IN THE AQUATIC ENVIRONMENT

At the time of drafting this disclosure document ((November 2002) Council Directive 99/45/EC is implemented as The Chemicals (Hazard Information and Packaging for Supply) Regulations 2002 - CHIP3. Recent adaptations to the Directive have been introduced into the U.K. as amendments to CHIP 3.

1.2.5 TYPICAL FRAME FORMULATION OF A YACHT ANTIFOULING PAINT

A typical yacht paint consists of biocide and pigment dispersed in a resinous binder, reduced to an acceptable application viscosity with solvent. Additives are incorporated to modify paint film properties, application or storage characteristics.

2. PHYSICAL CHEMISTRY

Identity, physical chemistry properties and analytical methods for N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulfamide have been summarised. Summarised test reports were provided. No QA or GLP compliance statements were supplied.

2.1 IDENTITY OF THE ACTIVE SUBSTANCE

BSI Name : dichlofluanid

IUPAC name : N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulfamide

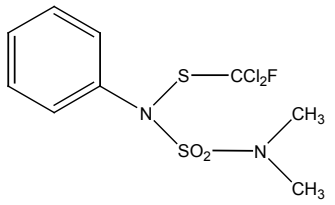
Other synonyms or codes : Euparen; KUE 13032C; Eluron; DFTS

CAS number : 001085-98-9

EINECS number : 214-118-7

Molecular formula : $C_9H_{11}Cl_2FN_2O_2S_2$

Structural formula :



Molecular mass : 333.2

Percentage purity : 98-99 % w/w (typical batch purity 98 % w/w)
*****_87.5 -92.5 % w/w (typical batch purity 90 % w/w)_ [Unpublished, 1993]

Spectral data :the spectral data provided (UV, IR, ^{1}H , ^{13}C NMR and MS) are broadly consistent with the chemical structure of the compound [Unpublished,1986(a)]

*****Figures in italics are values for Preventol A4-S, the stabilised active ingredient.

2.2 PHYSICO-CHEMICAL PROPERTIES

Physical state at 20 ^{o}C and 101.3 kPa :	colourless, crystalline solid [Unpublished, 1986(b)]
Melting/freezing point :	105 ^{o}C [Unpublished, 1986(b)]
Boiling point :	not provided
Relative density :	not provided
Bulk density :	400 kg m^{-3} (loose) [Unpublished, 1993]
Surface tension at 25 ^{o}C :	not provided
Vapour pressure at 25 ^{o}C :	3.79 x 10^{-5} Pa [Unpublished, 1988]
Solubility in water at 20 ^{o}C :	1.3 mg l^{-1} [Unpublished, 1985]
Partition coefficient: log Pow at 21 ^{o}C :	3.70 [Unpublished, 1987]
Flammability :	not flammable

2.3 STORAGE STABILITY

No data have been provided.

2.4 ANALYTICAL METHODS

The following acceptable methods for the determination of dichlofluanid have been submitted

i) analytical method for the determination of dichlofluanid in the technical material using reverse phase HPLC with UV detection at 254 nm. [Unpublished, 1983]

ii) analytical method for the determination of the impurities in the technical material using reverse phase HPLC with UV detection at 254 nm. [Unpublished, 1983]

iii) analytical method for the determination of dichlofluanid in water using GC-FID. [R. Brennecke and K. Vogeler, 1984]

iv) analytical method for the determination of dichlofluanid in formulations (other than water) :

a) TLC [Unpublished, 1990]
b) photometry [Unpublished, 1990]
c) GC-FID [Unpublished, 1990]

2.5 DATA REQUIREMENTS :

a) For technical dichlofluanid, measurements and full test reports for boiling point, relative density and surface tension.

b) Storage stability of representative current products under ambient conditions in the product packaging.

c) Details of an analytical method to determine dichlofluanid in water with a limit of quantitation of 0.1 $\mu g\ l^{-1}$.

3 MAMMALIAN TOXICOKINETICS AND TOXICOLOGY

3.1 MAMMALIAN TOXICOKINETICS

An adsorption, distribution, metabolism, elimination (ADME) study using ring labeled ^{14}C dichlofluanid (1.25 Mbq mg^{-1} and 99 % purity) is available, which was conducted to GLP. This study was sub-divided into single- and repeat- dose phases.

Dichlofluanid was administered by gavage to rats (Sprague Dawley, 5/group/sex). Two groups received either 2 or 20 mg kg^{-1}. A third group of animals was administered unlabeled ai for 14 days prior to a single oral dose of labeled ai (2 mg kg^{-1}). The amount of radioactivity remaining 48 h post dosing was determined in the following organs : carcass; carcass minus GI tract; bone (femur); brain; erythrocytes; fat (peri-renal); heart; kidney; liver; muscle; plasma; skin; spleen; and thyroid.

The following data apply to all groups. Elimination was rapid with 0.28-0.47 % of the dose remaining in the GI-less body 48 h post dose. Fifty percent of the administered dose was eliminated in the urine within 5-10 h and 90 % at 23 h. Renal clearance was reported to be 0.6-1.3 ml min^{-1} (GFR 0.8-1.5 ml min^{-1}), with some sex differences; see Table 3.1. Faecal excretion was between 3.1 and 8.6 % at 48 h. In males the faecal excretion component was found to be larger in both the repeat dose and at the single high dose (60 % and 38 % respectively) in comparison to females, although slight in absolute terms. See Table 3.2. The total recovery was >91 %.

Table 3.1 : Sex Differences In Renal Clearance (ml min^{-1})

sex	2 mg kg^{-1}	2 mg kg^{-1} (repeat dose)	20 mg kg^{-1}
male	1.29 ± 0.14	1.33 ± 0.07	1.02 ± 0.11
female	0.80 ± 0.05	0.89 ± 0.11	0.64 ± 0.05

Table 3.2 : Percentage Of Administered Dose Recovered From Urine And Faeces

	males			females		
	2 mg kg^{-1}	2 mg kg^{-1} rpt	20 mg kg^{-1}	2 mg kg^{-1}	2 mg kg^{-1} rpt	20 mg kg^{-1}
urine	89.1 ± 6.1	90.8 ± 4.5	90.4 ± 1.6	89 ± 6.3	96.4 ± 2.6	92 ± 2.1
faeces	7.2 ± 4.2	8.6 ± 2.8	6.9 ± 1.3	2.9 ± 0.9	3.4 ± 0.9	4.3 ± 0.9

Dichlofluanid was found not to accumulate in the carcass or carcass minus GI tract. Localisation of activity was confined to the liver and thyroids. No differences were reported between dosing regimens. [Unpublished, 1985(b)]

A subsequent study is available in which the faecal metabolites of ^{14}C-ring-labelled dichlofluanid (1.247 MBq mg^{-1}, 99 % purity) were investigated. Rats (2 male Sprague Dawley) were administered by gavage a single dose of dichlofluanid (10 mg kg^{-1}) and faeces

were collected over a 48-h period. Faecal samples were extracted using methanol. Metabolites were then identified directly or further extracted into a toluene/water system.

A further aliquot of the methanol extract was dried and digested with arylsulphatase and β glucuronidse to identify conjugates. Identification was carried out by gel permeation chromatography, mass spectrometry, thin layer chromatography or gas chromatography. Five identifiable faecal metabolites were recovered and potential routes of metabolism were identified; see Figure 3.1. Dichlofluanid was initially degraded to N,N-dimethyl-N-phenylsulphamide (DMSA). The DMSA was then subject to further biotransformation by a combination of ring hydroxylation and N-demethylation. The hydroxy metabolites were probably excreted as conjugates, based on enzymatic hydrolysis experiments using arylsulphatase and β glucuronidse. No quantification was provided, therefore it was not possible to distinguish between major and minor metabolites. [Unpublished, 1986(e)]

Two studies (male rats) using ^{14}C dichlofluanid (99 % purity) labeled at the fluorodichloromethyl sulphenyl group (FCl_2CS-) are available.

In the first study, animals (Sprague Dawley males, numbers not specified) received a single dose of ^{14}C-labelled dichlofluanid (2.16 MBq mg^{-1}) either 5 or 10 mg kg^{-1} by gavage or i.v.. Cumulative urine samples were collected over an 8-h period and assayed for both parent and metabolites by direct application to TLC plates or a reverse phase HPLC column. Of the administered dose at 10 mg kg^{-1}, 40-50 % of the label was recovered as TCC from either route after 8 h. At 5 mg kg^{-1}, 74-82 % and 57-64 % of the radiolabel was recovered from the i.v. and p.o. routes respectively after 8 h. The major urinary metabolite identified was thiazolidine-2-thione-4-carboxcylic acid (TCC). These data suggest cleavage of the FCl_2CS-group from the parent molecule, dichlofluanid. See Figure 3.2. [Unpublished, 1978(b)]

In the second study, animals (no information on strain, 5/group) were administered either 0, 0.1, 5, 10 or 20 mg kg^{-1} dichlofluanid (2.22 Mbq, 99.5 % purity) by gavage. A further group (5 animals) were administered a single i.v. dose of 10 mg kg^{-1} for whole body autoradiography. A final group of bile duct cannulated animals (5 animals) were administered 0.5 mg kg^{-1} to determine the extent of biliary excretion. The amount of radioactivity recovered in the expired air was determined for the 5 mg kg^{-1} oral dose only. It was found that 22 % of the dose was eliminated in expired air after 48 h, of which 90 % was eliminated in the first 8 h. The total radioactivity eliminated was independent of the applied dose. However, there was a shift from urinary to faecal excretion at the top dose. See table 3.3.

Table 3.3 : Percentage Of Radioactivity Excreted By Faecal And Urinary Routes (48 h)

dose (mg kg^{-1})	urine	faeces	total
0.1*	56±7	20±3	76
5	54±6	22±4	76
20	43±4	33±4	76

* n=4

Figure 1

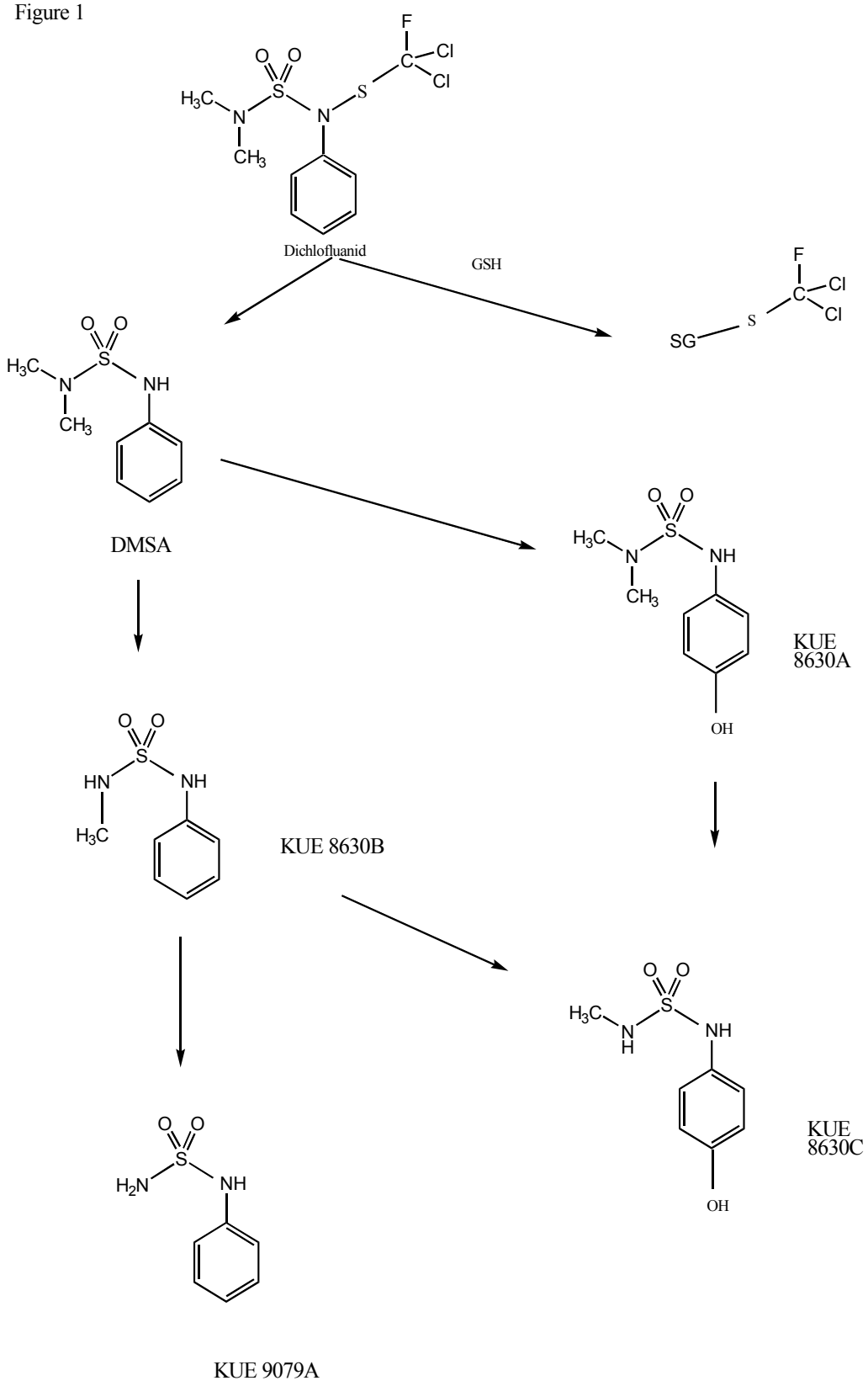


Figure 3.1 Proposed Metabolites of Dichlofluanid in Rat faeces.

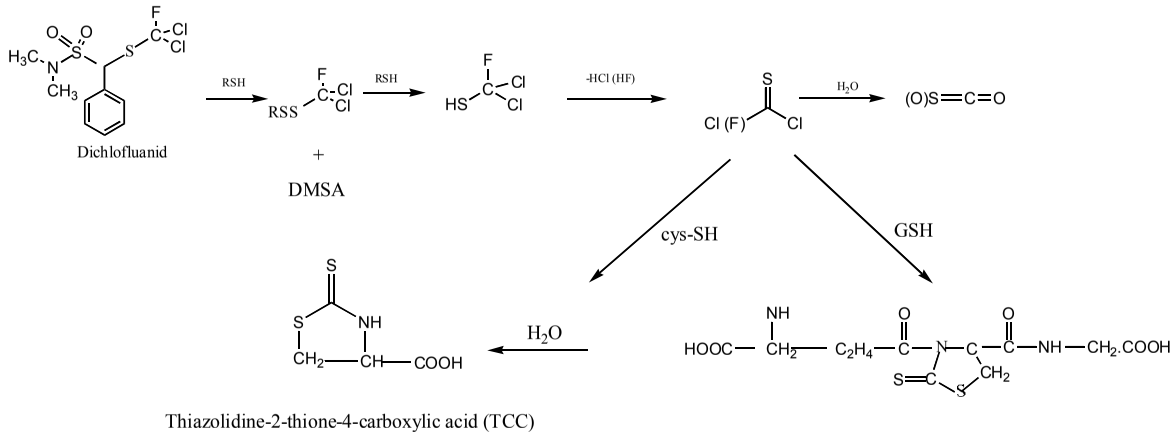


Figure 2

Fig 3.2 Proposed Metabolites of Dichlofluanid in Rat Faeces and Urine

Of the 20-30 % radiolabel found in the faeces, approximately 20 % remained unabsorbed since in bile duct cannulated animals 7 % was excreted in the bile. The amount of radioactivity in the body was followed over 10 d in animals receiving 5 mg kg^{-1}. Data provided on accumulation indicated that the thyroid accumulated radiolabel in comparison to other organs. See Table 3.4.

Table 3.4 : Relative Concentration* Of Radiolabel In Selected Tissues At 5 mg kg^{-1}

	time						
site	**2 h**	**8 h**	**1 d**	**2 d**	**3 d**	**6 d**	**10 d**
GI less body	21	6.3	2.7	2.1	1.6	1.2	0.8
blood	24	13	4.5	2.8	2.2	1.5	1
erythrocytes	23±4	17±1	5.2±10.3	3.2±0.3	2.2±0.1	2.1±0.2	1.7±0.1
thyroid	54±4	150±12	164±20	132±3	-	91±17	54±8
liver	37±7	15±0.4	7.6±0.4	4.9±0.5	3.8±0.4	2.4±0.4	1.1

*Relative concentration = measured activity / gramme of plasma or tissue
applied activity / gramme body weight

Note: to convert to ppm multiply by the dose in mg kg^{-1}

The autoradiograms were difficult to interpret but appear to confirm thyroid accumulation. The total recovery was >97 %. [Unpublished, 1977]

3.1.1 SUMMARY OF MAMMALIAN TOXICOKINETICS

No human data were available to address the toxicokinetics of dichlofluanid. No data were presented regarding the toxicokinetics of dichlofluanid following inhalation or dermal administration.

Four oral dosing toxicokinetic studies were available, all performed in the rat. The first investigated the ADME of ^{14}C ring labelled dichlofluanid. The second investigated the faecal metabolites of ^{14}C ring labelled dichlofluanid. A further two studies investigated the generation and fate of the fluorodichloromethyl sulphenyl group.

Following oral dosing, 80-90 % of the administered radiolabel was absorbed. Following absorption the radiolabel was widely distributed, with the principle locations being thyroid and liver. No data were presented which indicated that dichlofluanid would bioaccumulate. Dichlofluanid is initially metabolised, via non-enzymatic reactions with cellular thiols, such as cystein or glutathione (GSH) to yield N,N-dimethyl-N-phenylsulphamide (DMSA) and the fluorodichloromethyl sulphenyl moiety. The DMSA undergoes further hydroxylation and N-demethylation, and phase II conjugation reactions. The fluorodichloromethyl sulphenyl conjugate is further metabolised via reaction with GSH or cystein to eventually form thiazolidine-2-thione-4-carboxcylic acid (TCC). The amount of TCC generated was

apparently independent of route of administration, but less TCC was generated following high dose administration that at the low dose. This could be as a result of a secondary, low affinity high capacity pathway becoming involved. The principle route of elimination was via the urine for all metabolites. Following administration of dichlofluanid radiolabelled at fluoro- dichloromethyl sulphenyl group a shift from urinary to faecal elimination was noted at the top dose, a possible indication of depletion of cellular GSH pools. At 5 mg kg^{-1} 22 % of the radiolabel was detected in the expired air.

No evidence of toxicity was observed following acute dermal application, suggesting dichlofluanid does not cross the skin. It is apparent that dichlofluanid is systemically available following oral dosing. No acute or repeated dose toxicity information is available via the inhalation route of exposure, therefore no predictions can be made as to the toxicokinetics via this route.

The available data indicate that dichlofluanid is extensively and rapidly metabolised and these metabolites are eliminated via the urine, suggesting both a low molecular weight and high water solubility. It is likely that the toxicokinetics of dichlofluanid observed in the rat will be similar in humans.

3.2 DATA REQUIREMENTS : None

3.3 MAMMALIAN TOXICOLOGY

3.3.1 ACUTE TOXICITY

Of the acute oral, dermal and inhalation studies on the active ingredient summarised below, the first acute oral study in the rat, the inhalation study in the rat and rabbit dermal study were GLP compliant, the inhalation study in the rat and the rabbit dermal study were also carried out to EPA guidelines. Unless otherwise specified, the dichlofluanid used in the acute oral, dermal and inhalation studies on the active ingredient and formulations was of 90 % purity. For the irritation and sensitisation studies on the active ingredient, the purity of the dichlofluanid was not specified and unless otherwise stated these studies were not conducted to GLP standards.

3.3.1.1 Oral

3.3.1.1.1 Active Ingredient

In an acute oral toxicity study in the rat (SPF Wistar), animals were administered by gavage in water at 0, 1000, 2500 or 5000 mg kg^{-1} dichlofluanid (5/sex/group). No mortalities were reported. The LD_{50} was found to be >5000 mg kg^{-1}. Signs of toxicity reported were : soft faeces at the low dose, with apathy and piloerection reported at higher doses; laboured breathing; increased water intake and urine volume were also observed at the top dose only. These signs were reported from 2.25 h post dose and resolved by day 6 post dose. There was a transient drop in body weight gain in the top dose group and in one male and female at the middle dose. All body weights became comparable to control values by the end of the study. No treatment-related pathology was reported. A NOAEL was established at 1000 mg kg^{-1} based on signs of toxicity reported at higher doses. [Unpublished, 1990]

The acute oral toxicity of dichlofluanid was investigated in a number of species. In all studies the route of administration was by gavage and was of 14 d duration. The LD_{50} values were : >5000 mg kg^{-1} in both fasted and non-fasted male and female rats; 5464 and 5597 mg kg^{-1} in male and female mice respectively; 954 mg kg^{-1} in female guinea pigs; and 3500 mg kg^{-1} in female rabbits. The signs of toxicity reported, apathy, dyspnoea and sedation were similar in all species and were resolved prior to study termination. Necropsy of decedents found affected organs to be : the spleen, kidney and stomach in fasted rats; the kidney, liver and GI tract in mice; the liver, kidney and spleen in female guinea pigs; and the liver kidney and stomach in female rabbits. No abnormal pathologies were reported in animals examined at study termination. [Unpublished, 1978(c)]

3.3.1.1.2 Formulations

Animals (Wistar rats 4/sex/group) were administered by gavage either 3.1, 6.2, 12.5, 25 or
50 ml kg^{-1} of a mineral oil-based formulation (containing 0.4 % w/w dichlofluanid, 88 % purity). Animals were observed for 14 d post dose. No deaths were reported below 12.5 ml kg^{-1}. One animal receiving 12.5 ml kg^{-1} died on day 3, 7/8 died between days 2-5 at 25 ml kg^{-1}, and all animals died at the top dose by day 3. The signs of toxicity varied with the dose received and included slight diarrhoea and nasal discharge which resolved after 1 d at 3.1 and 6.2 ml kg^{-1}. At 12.5 ml kg^{-1} the signs reported were diarrhoea, salivation and slight ataxia

which persisted for 2 d post dose. At 25 ml kg^{-1}, ataxia, paralysis of the hind limbs, gasping, urinary stains and serosanguinous discharge around the nose and mouth were reported. At the top dose excessive salivation, lacrimation and ataxia were reported, with the animals becoming comatose. The onset of symptoms began 30 minutes post dosing and became progressively more severe. The LD_{50} was calculated to be 16.5 ml kg^{-1}. No further information was provided. The NOEL for this formulation was 6.2 ml kg^{-1}; equivalent to 66 mg kg^{-1}. From this study, the NOEL in terms of dichlofluanid is 24.8 mg dichlofluanid kg^{-1}. [Unpublished, 1980]

3.3.1.2 Dermal

3.3.1.2.1 Active Ingredient

In a briefly reported study, dichlofluanid (0 or 5000 mg kg^{-1}) was applied under an occlusive dressing to the shorn skin of rats (5 sex/group) for 24 h. The LD_{50} was found to be >5000 mg kg^{-1}. No further information was provided. [Unpublished, 1988]

In an acute limit study in the New Zealand White rabbit (5/sex) using a 24 h occluded patch test, the LD_{50} was >2000 mg kg^{-1}. The reported composition included 7.5 % inert co-formulants and 2.5 % impurities. No mortalities or signs of toxicity were reported at this dose. The NOEL was >2000 mg kg^{-1} for both sexes. [Unpublished, 1986(a)]

3.3.1.2.2 Formulations

Animals (Wistar rats 5/sex/group/formulation) received a single dermal application of 2.5, 3.5, 5 or 7.5 ml kg^{-1}, of one of two organic solvent-based formulations (containing 0.4 % or 1 % dichlofluanid, 88 % purity) to both intact and abraded skin. No deaths were reported throughout the 14-d study period for either formulation. The signs of toxicity, onset and duration were the same for both formulations. At the lowest dose signs of toxicity were piloerection and ptosis, beginning 60 minutes post application and resolving after 24 h. Piloerection, apathy and ptosis were reported at 3.5 ml kg^{-1}, beginning 60 minutes post application and continuing for 24 h. At 5 ml kg^{-1} tremor, ptosis, piloerection and apathy were reported. These effects were reported from 60 minutes post application and resolved by day 3. At 7.5 ml kg^{-1} tremor, ptosis, piloerection, slight lacrimation, ataxia and bradypnoea were reported from 60 minutes post application and resolved by day 3. No further information was provided. The NOAEL was established at 2.5 ml kg^{-1} based on transient signs of toxicity reported at 3.5 ml kg^{-1}; equivalent to 14 and 22 mg kg^{-1} at 0.4 and 1 % respectively . [Unpublished, 1981]

3.3.1.3 Inhalation

3.3.1.3.1 Active Ingredient

Sprague Dawley rats (10/sex/group) were exposed (head only) for 4 h to an aerosol containing, 0, 1088, 1969, or 2529 mg m^{-3} of dichlofluanid (88.5 % purity). Females were also exposed to an additional dose of 773 mg m^{-3}. The reported composition included co-formulants and 2.5 % impurities. Animals were observed for 14 d post dose prior to sacrifice. The MMAD was 3.5-4.7 μm (4 h) and 5 μm (1 h). Deaths were reported at all doses in both

sexes from days 0-3 post dose. In males the mortalities were 3/10, 8/10 and 10/10 at 1088, 1960 and 2569 mg m^{-3} respectively. In females the following number of deaths were observed : 4/10; 4/10; 5/10; and 9/10 at 773, 1088, 1969 and 2569 mg m^{-3} respectively. Weight gain was affected in males at 1088 and both sexes at 1969 mg m^{-3}. However, this became comparable to controls at study termination.

Determination of the effects upon weight gain in the top-dose group could not be made as only one animal survived past day 2. Those animals dying during the study were found to have: nasal, ocular, oral and generalised facial and ventral thoracic stains; wet red or minimally firm lungs; red turbinates and ocular opacity. No treatment-related lesions were reported at the end of the study. The calculated LC_{50} values at 4 h were 1338 mg m^{-3} (male) and 1233 mg m^{-3} (female). It was not possible to determine a NOEC in this study as deaths were reported at all doses. These data meet the criteria for classification of dichlofluanid as harmful by inhalation.

A 1-h exposure (head only) using 0, or 2469 mg m^{-3} of dichlofluanid is also available. The number of animals, strain and active ingredient were the same as in the 4 h exposure. Two females died during the study period. With the exception of alopecia, signs of toxicity and observations on decedents were similar to those reported in the 4-h study. All symptoms had resolved by day 8, except for the alopecia. No abnormal pathology was reported in animals sacrificed at the end of the study. The LC_{50} value was >2469 mg m^{-3} and the NOEC at 1 h was <2469 mg m^{-3}, due to signs of toxicity and deaths at the only dose used. [Unpublished, 1986(c)]

3.3.1.3.2 Formulations

No acute inhalation data on formulations has been provided.

3.3.1.4 Skin Irritation

3.3.1.4.1 Active Ingredient

In a skin irritation study, 500 mg of dichlofluanid (water-based paste) was applied under an occluded patch for 4 h to the abraded and non-abraded skin of 6 New Zealand White rabbits. Animals were observed for 7 d post treatment, with scoring for erythema and oedema at 24 and 72 h only. Both oedema (mean 0.4, maximum 1) and erythema (mean non-abraded 1, maximum 2) were reported. Erythema had resolved by 72 h, but 50 % of animals showed slight oedema (grade 1) at 72 h. No further information was provided. Dichlofluanid was not classified as a skin irritant. [Unpublished, 1982(c)]

3.3.1.4.2 Formulations

An organic solvent-based dichlofluanid-containing formulation (0.5 ml, containing 0.4 % dichlofluanid of 88 % purity) was applied under an semi-occluded patch for 24 h to abraded and non abraded skin of 6 male New Zealand White rabbits. Animals were observed 24 and 72 h

post treatment only. The grades for the intact skin were erythema (mean 0.75, maximum 2) and oedema (mean 0.3, maximum 2). The grades for the abraded skin were erythema (mean 1.4, maximum 3) and oedema (mean 1.1, maximum 1.1). The formulation was not classified as a skin irritant. It should be noted that the duration of exposure is unclear. [Unpublished, 1980]

The following studies were GLP compliant and conducted according to Directive 84/449. In each study, 3 male New Zealand white rabbits were used.

An organic solvent-based dichlofluanid-containing formulation (0.5 ml, containing 0.4 % active ingredient of 88 % purity) was applied under a semi-occlusive patch for 4 h. Animals were observed 30 minutes post treatment and subsequently every 24 h for 4 d. Both oedema and erythema (grade 1) were reported after 30 minutes in all animals. After 24 h only one animal was still symptomatic (grade 1). All effects had resolved at 48 h post treatment. The formulation was not classified as a skin irritant. [Unpublished, 1991]

In a second study an organic solvent-based dichlofluanid-containing formulation (0.5 ml containing 0.4 % active ingredient of 88 % purity) was applied under a semi-occlusive patch for 4 h. Animals were observed 30 minutes post treatment and subsequently every 24 h for 4 d. Both oedema and erythema (grade 1) were reported after 30 minutes in all animals. After 24 h post treatment 2/3 animals were still symptomatic, with complete resolution after 48 h. The formulation was not classified as a skin irritant. [Unpublished, 1991]

3.3.1.5 Eye Irritation

3.3.1.5.1 Active Ingredient

In an eye irritation study, dichlofluanid (1 mg) was instilled into the conjunctival sac of one eye of six New Zealand White rabbits, with the other eye serving as a control. No information was provided at 48 h. No effects were reported in the iris at either 24 or 72 h. One incidence of slight corneal opacity (maximum score, 1) was reported. Conjunctival redness (mean grade 2.7, maximum 3) and swelling (mean grade 1.5, maximum 3 at 72 h) were reported with effects persisting (maximum grade 1) up to 7 d. All effects resolved by the end of the study on day 14. Dichlofluanid was classified as an irritant. [Unpublished, 1982(c)]

3.3.1.5.2 Formulations

An organic solvent-based dichlofluanid-containing formulation (0.1 ml, containing 0.4 % active ingredient of 88 % purity) was instilled into the conjunctival sac of one eye of each of three animals, with the other eye serving as a control. Observations were made at 1, 24, 48,72, 96 h and at 7d post treatment. No iris effects were reported. Conjunctival chemosis (grade 1) and redness (2 animals at grade 1 and 1 animal at grade 2) were reported after 30 minutes, along with a slight loss of corneal epithelial cells (grade 0). The conjunctival redness was evident in 2 animals at 24 h and in 1 animal at 48 h. The chemosis had resolved after 24 h and the redness after 48 h. The formulation was not classified as an eye irritant. [Unpublished, 1991]

In a second eye irritation study an organic solvent-based dichlofluanid-containing formulation (0.1 ml, containing 0.4 % active ingredient of 88 % purity) was instilled into the conjunctival sac of one eye of three animals, with the other eye serving as a control. Observations were made at 1, 24, 48,72, 96 h and at 7d post treatment. No effects were reported in the iris. Conjunctival chemosis (grade 1) and redness (2 animals grade 1 and 1 grade 2) were reported after 30 minutes along with a slight loss of corneal epithelial cells (grade 0). At 24 h post treatment one animal was found with conjunctival redness (grade 1) and a second still exhibited a slight loss of corneal epithelial cells. All effects had resolved after 48 h. The formulation was not classified as an eye irritant in this study. [Unpublished, 1991]

None of the following studies, carried out with dichlofluanid containing formulations, were GLP compliant.

An organic solvent-based dichlofluanid-containing formulation (0.1 ml, containing 0.4 % dichlofluanid of 88 % purity) was instilled into the conjunctival sac of one eye of each of three male New Zealand white rabbits, with the other eye serving as a control. Observations were made at 24, 48, 72 h and at 7 d post treatment only. No effects were reported in the iris or cornea. The mean grades (at 24, 48 and 72 h) for erythema, chemosis and discharge were 1.5, 1.3 and 1.5 respectively with the maximum being 3 in each case. These effects did not persist after 72 h. The formulation was not classified as an eye irritant. [Unpublished, 1980]

3.3.1.6 Sensitisation

3.3.1.6.1 Active Ingredient

In a Magnusson and Kligman guinea pig maximisation test, induction and challenge concentrations were selected following a pilot study which showed that applications of 12.5 and 25 % dichlofluanid were non-irritating, with 50 % being minimally irritating. In the induction phase, animals (male Pirbright albino, 15/group) received 3 single intradermal injections to separate sites of : 0.1 ml 50 % aqueous solution of Freunds adjuvant; 0.1 ml of
10 % aqueous dichlofluanid; and 0.1 ml of 10 % dichlofluanid in Freunds adjuvant. These were followed one week later by topical application of 5% aqueous dichlofluanid under an occlusive dressing for 48 h. Controls were similarly treated but with water replacing dichlofluanid. No adverse reactions were reported during the induction phase. Two weeks later animals were challenged with an application of the test substance under an occluded dressing (12.5 % left flank or 25 % right flank) for 24 h and examined after 24 and 48 h. Table 3.5 shows percentages of positive animals.

Table 3.5 : Percentages Of Animals Responding After Dichlofluanid Challenge

12.5 % dichlofluanid				25 % dichlofluanid			
24 h		48 h		24 h		48 h	
control	treated	control	treated	control	treated	control	treated
6 %	73 %	-	87 %	-	87 %	6 %	87 %

Examination of control data found two incidences of grade 1 erythema only. The mean grades reported for 12.5 % dichlofluanid were 0.86 and 1.3 at 24 h (2/15 grade 2, 9/15 grade 1) and 48h (6/15 grade 2, 7/15 grade 1) respectively, and for 25 % dichlofluanid were 1.4 and 1.5 at 24h (9/15 grade 2, 4/15 grade 1) and 48h (7/15 grade 2, 6/15 grade 1) respectively. The maximum was grade 2 in each case. The criteria for a grade 1 was "slight patchy redness" and grade 2 "moderate diffuse redness".

Histopathology of treated areas found that some animals had a slight to moderate intracellular oedema of the *stratum corneaum* and *stratum granulosum*. The epidermis was thickened in these animals and the corneaum areas around the papillae showed moderate to severe round cell infiltration. Dichlofluanid is classified as a sensitiser, as there was a >30 percent increase in responding animals compared to controls. [Unpublished, 1980(a)]

A Draize sensitisation study is available using male Pirbright albino guinea pigs, 15/group. During the induction phase, animals received intradermal injections of 0.1 % aqueous dichlofluanid (1 x 0.05 ml and 9 x 0.1 ml) in a series of 10 injections (one on each day on Monday, Wednesday and Thursday of three consecutive weeks and the beginning of the fourth week). Observations were made 24 h after each injection. Fourteen days after the final injection animals were challenged with a single intradermal injection of 0.05 ml of 0.1 % aqueous dichlofluanid. The mean grades, post challenge for erythema were : control 1, 15/15 grade 1; treated 3.3, 5/15 grade 3; and 10/15 grade 4. The grades for oedema were determined by direct measurement of the affected area (control 0.43 cm and treated 1.1 cm respectively). The applicant considered dichlofluanid to be a sensitiser on the basis of these data. [Unpublished, 1980(b)]

A Klecaks open epicutaneous test is available, carried out in the guinea pig (female Pirbright albino, 7/group test plus control). Animals received 20 dermal applications (5/week for 4 weeks) of 1 %, 3 %, 10 % or 30 % (dichlofluanid 90 % purity) in water on shorn skin. These concentrations were chosen following a range-finding study in which a single exposure to a 25 % aqueous suspension was found to be non-irritating. A further range-finding study was carried out with dichlofluanid in peanut oil which found 3.1 % dichlofluanid was irritating, but was not further considered. Skin reactions were reported 24 h after each treatment during the induction phase. Mild erythema (maximum grade 1) was reported in a number of animals beginning after the seventh application with 1-10 % suspensions and after the sixth application with the 30 % suspension.

Challenge took place at 4, 6 and 8 weeks after the start of induction with concentrations of 3 %, 10 % or 30 % aqueous suspensions, and 100 % dichlofluanid. Animals were observed 24 and 48 h after treatment. After the first challenge mild erythema (maximum grade 1) was observed in the majority of animals receiving induction concentrations of >3 % (at 3 % effects were noted at 48 h only). After the second challenge similar responses were also reported in animals receiving challenge concentrations of 1 %, and one control animal was also positive. After the third challenge there was no increase in severity. However, instances of positive reactions in control animals (1-3) were reported at each induction concentration; grade 0.5 only. Animals receiving 100 % dichlofluanid either responded poorly or were negative after each challenge. This may have been due to the dichlofluanid being applied as a solid. The study authors concluded from this study that dichlofluanid was a sensitiser.

However as this study will not be used for classification purposes, these data can be treated with some caution. [Unpublished, 1980(d)]

3.3.1.1.2 Formulations

No sensitisation data on formulations have been provided.

3.3.1.7 Summary Of Acute Mammalian Toxicity

3.3.1.7.1 Active Ingredient

An acute oral NOEL was established in the rat of >1000 mg kg^{-1}, with an LD_{50} of >5000 mg kg^{-1}. The female guinea pig and female rabbit were more sensitive, and mice slightly less sensitive, than the rat to an acute oral dose of dichlofluanid. The dermal LD_{50} was >5000 mg kg^{-1} in the rat. Inhalation exposure in the rat (1 h, head only) found the LC_{50} to be >2469 mg m^{-3} and the NOEC <2469 mg m^{-3}. A four-hour exposure (head only), carried out in the rat found the LC_{50} to be 1338 and 1233 mg m^{-3} respectively. A NOEC was not determined as deaths were reported at all doses. Inhalation exposure in the rat was found to be the most sensitive acute toxicity end-point. Dichlofluanid is classified as harmful by inhalation.

Dichlofluanid was not classified as a skin or eye irritant. Three sensitisation studies were carried out : a Magnusson and Kligman guinea pig maximisation test; a Draize sensitisation study; and a Klecaks open epicutaneous test. The Magnusson and Kligman test was positive. Both the Draize sensitisation study and Klecaks open epicutaneous test were considered by the study authors to be positive. On the basis of these three studies it was concluded that dichlofluanid should be classified as a sensitiser.

3.3.1.7.2 Formulations

The eye and skin irritation potential of two organic solvent- and one spirit-based dichlofluanid -containing formulations (containing 0.4 % dichlofluanid of 88 % purity) was assessed. None of these formulations was classified as an irritant. An acute oral toxicity found the NOEL of an organic solvent-based dichlofluanid-containing formulation (containing 0.4 % dichlofluanid of 88 % purity) to be 6.2 ml kg^{-1}, equivalent to 66 mg kg^{-1}. The acute dermal NOAELs of two spirit-based formulations containing dichlofluanid (containing 0.4 % or 1 % dichlofluanid of 88 % purity) were 2.5 ml kg^{-1}; equivalent to 14 and 22 mg kg^{-1} respectively. These data indicated the formulations to be more toxic than the active ingredient, by both the oral and dermal route.

3.3.2 REPEAT-DOSE TOXICITY

A single repeat-dose study carried out in the rat is available. This study was not conducted to a recognised protocol or to GLP standards. Three repeat-dose studies carried out in the dog are also available.

3.3.2.1 Rat

In a 4-month study in the rat (FB 30 strain, 15/group/sex), animals received dietary administration of either 0, 100, 300 or 1000 ppm dichlofluanid (92 % purity). The study was repeated using 0, 3,000 and 10,000 ppm as no definite signs of toxicity were observed. All data provided are from the second study. Haematology and urinalysis data were obtained at necropsy only. Deaths were reported at 3000 ppm (1 male and 1 female); at 10,000 ppm
11 animals died prior to study termination (from day 7, 5 males and 6 females). Animals in the 10,000 ppm group exhibited a decrease in food consumption (21 % and 23 % males and females respectively) and body weight gain (19 % and 10 % males and females respectively) throughout the study period. No effects on haematological parameters or urinalysis data were reported; data on clinical chemistry data was not provided.

In males the absolute heart weight was significantly decreased at 3,000 ppm (10 %) and
10,000 ppm (12 %), with a decrease in adrenal gland weight at 10,000 ppm only (22 %). In females the absolute liver weight was significantly increased at 3,000 ppm (22 %) and 10,000 ppm (24 %) with a decrease in adrenal weight (28 %) at 10,000 ppm only. Histopathology of kidney, liver, and spleen found unspecified "fine changes" in structure at 10,000 ppm. Hepatocytes in the 10,000 ppm group were enlarged and vacuolated, some with pyknotic nuclei and a loss of basophilic staining. Protein casts were found in the proximal tubule of kidneys in the 10,000 group, with some increase in reabsorption, suggested by an increase in the number of protein droplets in the proximal tubules. There was a decrease in lymphatic tissue in the spleen at 10,000 ppm with reduction of the follicle and lymphatic areas of the red pulp.

In this study a NOEL was not established. However, the LOAEL was 3,000 ppm; estimated to be equivalent to 180 and 341 mg kg^{-1} d^{-1} for males and females respectively. This was based on an increase in liver weight in females and a decrease in heart weight plus a decrease in body weight in males at this dose. [Unpublished, 1964]

3.3.2.2 Dog

3.3.2.2.1 Four-month Study

In a 4-month study, beagles (2/group/sex) were administered either 0, 500, 1500 or 4500 ppm dichlofluanid (90.2 % purity) in the diet. Both males (day 98 and 107) and one female (day 105) in the top-dose group died prior to study termination. In the top-dose group males appeared weak and females were inactive. Body weight gain (32 % males and females combined) and food consumption (32 %) were significantly reduced at the top dose.

Clinical chemistry findings were indicative of liver and kidney effects with ALT and AST elevated at 1500 ppm in one female, elevated ALP (222 %) and blood urea (97 %) at 4500 ppm (1 female only). However, these findings were not statistically significant as they were obtained from only one animal. No further information was provided. Based on effects noted

in one female, the NOEL was 500 ppm; estimated to be equivalent to 16.1 mg kg^{-1} d^{-1}. [Unpublished, 1966]

3.3.2.2.2 One-year Study

A repeat-exposure study using Beagles (4/dose/sex) is available; it was GLP and OECD Annex V compliant. Animals were administered either vehicle or 2.5, 12.5 or 37.5/62.5 mg kg^{-1} d^{-1} of dichlofluanid (90 % purity) in capsule form for one year. The top dose was reduced at week 15 to 37.5 mg kg^{-1} d^{-1} because of excessive toxicity. As a NOEL could not be established with the initial dosing regime, a subsequent study was carried out in which 0 or 1.25 mg kg^{-1} d^{-1} dichlofluanid was used. Interim blood samples were taken at 13, 26, 39 and 52 week (terminal bleed); in addition samples were taken from some animals in the top-dose group at 14, 15 and 19 weeks. At these time points clinical chemistry and haematological analysis was carried out. Urinalysis was carried out at 14 weeks, 28 weeks and at study termination. It is of note that as the control animals in the second study were shared with an unrelated study, the vehicle control was administered in the diet.

One female receiving 62.5 mg kg^{-1} d^{-1} was killed *in extremis* at week 15 prior to dose reduction. Necropsy of this animal revealed : the colon and small bowel were fluid filled; intestines were of an abnormal consistency; the oesophagus and stomach were of an abnormal consistency and texture; the liver was of an abnormal texture consistency and colour; and the thymus and thyroid were small. No further information was provided.

Food consumption was decreased at 62.5 mg kg^{-1} d^{-1} (36 % and 40 % males and females respectively at week 13) but became comparable to control values after dose reduction. A compound-related decrease in body weight was observed in males 12.5 and 37.5 mg kg^{-1} d^{-1} (6.4 % and 10 %) and females at 37.5 mg kg^{-1} d^{-1} (18 %). Reported clinical signs of toxicity in animals at 37.5 mg kg^{-1} d^{-1} were : sporadic instances of decreased activity; dehydration; rough coat; red gingivae from week 13; and increased salivation at week 9 only. Additional signs of toxicity reported in males were inflammation of the ears (top dose), sporadically from week 13, and red gingivae (middle dose), sporadically from week 32. No opthmological effects were reported.

The haematology findings in males receiving 37.5 mg kg^{-1} d^{-1} from 6 months onwards were decreased numbers of erythrocytes (4 %), haemoglobin concentration (7.5 %) and haematocrit (7.4 %). Mild to moderate hepatic haemosiderin deposition was reported and was particularly extensive in two animals. None of these effects achieved statistical significance. However, as they were outside the range of supplied historical control data they were considered to be treatment related.

The following data, in animals at the top dose, outside the range of supplied historical control data (±2SD), except BUN, creatinine, and triglyceride levels in females. This clinical chemistry data refers to the first study and unless otherwise stated the data were collected at study termination. Increases in ALP (215 % and 18 % males and females respectively), AST (81 % males only*), ALT (595 % and 293 % males and females respectively) and gammaGT (360 % males only) levels were reported in animals 37.5 mg kg^{-1} d^{-1}. An increase in ALT levels (350 % males only) at 12.5 mg kg^{-1} d^{-1} was also reported. Serum concentrations of cholesterol were elevated in animals receiving 12.5 mg kg^{-1} d^{-1} (20 % and 23 % males and

females respectively) and 37.5 mg kg^{-1} d^{-1} (132 %* and 89 % males and females respectively). The serum concentration of triglycerides were elevated in females only (63 % and 59 % at 12.5 mg kg^{-1} d^{-1} and 37.5 mg kg^{-1} d^{-1} respectively). Elevated BUN (males only 114 %) and creatanine levels were reported (77 %* and 22 %* males and females respectively) at 37.5 mg kg^{-1} d^{-1}. One male receiving 12.5 mg kg^{-1} d^{-1} was also reported to have elevated BUN and creatanine levels (*statistically significant). Levels of the thyroid hormones T3 (28 %) and T4 (37 %) were elevated at study termination in animals receiving 37.5 mg kg^{-1} d^{-1}.

At necropsy, treatment-related pathology findings were reported in males receiving 37.5 mg kg^{-1} d^{-1}. Externally one animal was pale with discoloration of the kidney, liver and thyroid which was also reduced in size. There were also non-significant decreases in both absolute and relative thyroid weights (39 % and 33 % respectively) and testes weights (25 % and 15 % respectively). Two animals were found to have bilateral testicular degeneration; no control animals were reported with bilateral testicular degeneration. In females absolute
(15-60 %) and relative (25-150 %) ovary weights were increased in all treatment groups. These increases were not dose related or statistically significant.

Treatment-related histopathology was reported in several organs. Changes were found in the kidney at the middle and top dose in females and in the top dose in males. These were described as minimal to moderate chronic nephropathy, and were chiefly characterised by proximal tubule nephrosis (4 males, 3 and 4 females, controls 0). In the liver at the top dose the following histopathological findings were reported : mild to moderate hepatocellular degeneration (2 and 4 females and males respectively); minimal to moderate haemosideriosis (males and females 2); and minimal to moderate periportal chronic-active inflammation with biliary hyperplasia (males and females 2). The controls were negative for each of these hepatic findings. Minimal to severe thyroid follicular cell degeneration was reported in all animals from the top-dose group and one male from the middle-dose group. The pituitary glands of several animals from the top-dose group were found to have mild to severe hyperplasia (2 males and 2 females) of large pale staining cells (basophils) in the *pars distailis*, among pituitary basophils are the thyrotrophs. Testicular degeneration was noted in 2 animals receiving 37.5 mg kg^{-1} d^{-1}, the lesion was widespread and of moderate/severe grade. Thymic atrophy was also reported in males receiving 37.5 mg kg^{-1} d^{-1} (3/4 animals).

In the second study (0 or 1.25 mg kg^{-1} d^{-1}), no obvious treatment-related effects were reported in the clinical chemistry, haematology or urinalysis parameters measured.

At necropsy the following findings were reported. Both absolute and relative testes weights were decreased (29 % and 25 % respectively). However in the absence of a dose response (when compared to the higher doses used in the first study), and as the weights were within the range of historical control values, this observation was not thought to be of toxicological significance. No statistically significant treatment-related organ weight changes were reported in females. Inspection of the data revealed absolute and relative ovary weight increases (60 % and 175 % respectively). The large SD values and small numbers make it difficult to assess the significance of these data. One incidence of testicular degeneration was reported at necropsy in both control and treated animals.

Overall the ACP concluded that the ovarian weight increases were not relevant and that a NOAEL of 2.5 mg kg^{-1} d^{-1} could be determined from these studies. [Unpublished, 1992]

3.3.2.2.3 Two-year Study

The following study is considered to be flawed due to the lack of histopathological evaluation and it was not GLP compliant. Beagles (4/sex/group) received dietary administration of either 0, 100, 300, 1000 or 3000 ppm dichlofluanid (90 % purity); equivalent to 3.6, 10.5, 34 and
107 mg kg^{-1} d^{-1} (males) and 3.4, 11, 34 and 101.4 mg kg^{-1} d^{-1} (females) respectively, for a two year study period. Interim blood sampling was carried out at 6 weeks, 6 months and 12 months for haematology, with interim clinical chemistry analysis being performed at 12 months.

One death was reported; a female in the top-dose group died of pneumonitis. Necropsy did not reveal any treatment-related pathology. No effects on food consumption or body weight were reported up to dose levels of 1000 ppm. Both food consumption and body weight gain were decreased at 3000 ppm. The weight reductions reported at necropsy were 18 % (males) and 25 % (females). Animals in the 3000 ppm dose group were also less active, often refused food, and had dull, ruffled fur.

No effects were reported on the haematological parameters investigated as part of the study. Changes reported in clinical chemistry parameters at 3000 ppm including increased ALP, ALT, bilirubin, cholesterol levels and BSP retention time, indicative of impaired hepatic function.

Against a background of variable data, increases in serum creatinine (27 % males and 33 % females, at 3000 ppm) and urea levels (34 % males and 50 % females, at 3000 ppm) were reported, this being suggestive of impaired renal function. Direct measurements of kidney function at study termination revealed a decrease in both p-aminohippuric acid (PAH) and inulin clearance; (5 and 17 %) in males and (19 and 32 %) females at 24 months at 3000 ppm. These data provide further evidence for impaired renal function.

Absolute organ weights in males of the liver, lung, spleen, kidneys, adrenals, pancreas and heart were all decreased at 3000 ppm; the greatest being a 35 % decrease in testes weight. Decreases in relative organ weights were reported in males in the thyroid, heart, spleen and testes (20 % at 3000 ppm) at the top dose. The relative kidney and liver weights were elevated at both 1000 (21 and 3.4 %) and 3000 ppm (18 and 10 %). In females absolute organ weights were reduced : the thyroid in all treatment groups, by 32-36 %; ovaries 31-55 % from 100 ppm; and heart by 14-27 % at 1000 and 3000 ppm. The absolute spleen weights were raised in females at 1000 ppm and above by 18-20 %. In females there was a slight increase in the relative organ weights of the pancreas above 1000 ppm and spleen weight was elevated by 25 and 60 % at 1000 and 3000 ppm. A decrease in ovary weight was reported at all doses (55 % at 3000 ppm). The changes in organ weight in females preclude the setting of a NOEL. [Unpublished, 1969]

3.3.2.3 Summary Of Repeat-Dose Toxicity

Dichlofluanid has been tested for repeat-dose toxicity in the rat, in a 4-month rat dietary and two lifetime studies, in two mouse lifetime studies, in 4-month and 2-year dog dietary studies and two 1-year capsule studies. No studies were presented with inhalation or dermal administration.

In longer term studies (summarised in 3.3.4 and 3.3.5) the most consistent findings were cranial osteosclerosis in the rat and findings consistent with fluorosis in a 2-year mouse study. In a 1-year dog study, clinical chemistry findings with supportive histopathology were observed, indicative of renal and liver damage. In this study a NOAEL of 2.5 mg kg^{-1} d^{-1} was established.

A detailed assessment of cranial osteosclerosis was performed in a rat 2-year study (summarised in section 3.3.5.1) in which a LOEL was established at 10-14 mg kg^{-1} d^{-1}. A clear increase in the incidence of cranial osteosclerosis was observed in the low- and middle-dose groups, with almost all animals affected at the top dose. These findings are likely to be secondary to fluorosis in these animals. In terms of interspecies comparisons, in a mouse 2-year study, thickening of both the appositional bone of the cranial vault and nasal turbinates, and tooth alveolitis were observed at the top dose of 1,731-1,873 mg kg^{-1} d^{-1}. These findings are also considered to be a secondary consequence of fluorosis in these animals. No evidence of fluorosis was observed in the dog studies. It is considered that these findings are likely to represent a fluoride mediated perturbation of bone metabolism but are not considered to be of concern for human health. It is considered that the observed liver and renal damage is of concern to human health.

3.3.3 GENOTOXICITY

3.3.3.1 *In Vitro* Studies With Bacterial Cells

Three *Salmonella typhimurium* bacterial point mutation tests are available, none were conducted to GLP. The *S. typhimurium* strains used were TA 1535, 1537, 100 and 98. An S9 fraction was prepared from the livers of aroclor 1254 induced male Sprague Dawley rats. The active ingredient was dissolved in dimethylsulfoxide (DMSO) and controls were dissolved in water. A doubling of the number of revertants was considered to be a positive response. With the exception of the second study, these were broadly Annex V compliant. This study was non-compliant because of inappropriate selection of a positive control substance for use in the absence of an S9 fraction.

In the first Ames test, concentrations in the range of 0-2500 µg/plate (100 % purity dichlofluanid) were chosen initially (4 plates/dose/strain). These were carried out in the presence of an S9 fraction only. A mutagenic effect was reported with TA 100 only at 100 µg/plate. Above this dose dichlofluanid was bacteriotoxic (82 % cell death at 500 µg/plate). To characterise this effect further, separate repeat experiments were carried out with TA 100 using concentrations from 0-400 µg/plate with S9 and 0-50 µg/plate without S9. With S9 bactericidal activity was reported at ≥100 µg/plate, with a 77 % at 100

and 200 µg/plate respectively (96 % at 400 µg/plate), with a 1.3x increase in the number of revertants, in the first repeat. In the second repeat carried out with S9, an increase in the number of revertants was reported at 50 and 100 µg/plate (1x and 2x respectively), bacteriotoxicity was reported at 50 (33 %) and 100 (45 %) µg/plate respectively (96 % at 400 µg/plate). No effects were reported in the absence of S9.

The positive control endoxan produced a 2-2.7x increase in the number of revertants. The positive responses in the repeat experiments were observed in the presence of cytotoxicity, and the increase in revertants less than that required for a positive result. Therefore, dichlofluanid can be considered negative. [Unpublished, 1979(a)]

In a second poorly conducted Ames test, an initial experiment was carried out with concentrations in the range 0-2500 µg/plate, except TA 100 (0-500 µg/plate). The purity of the dichlofluanid was 90 %. TA 100 was tested at all dose levels with and without S9. The three other strains were tested with S9 at all dose levels and without S9 at 100 and 2500 µg/plate. The positive controls used were endoxan for strains TA 1535 and TA 100, and trypflavine for TA 1537 and TA 98. An increase in the number of revertants was reported with TA 100 only, at 100 µg/plate with (1.5x) and at 20 µg/plate without S9 (2x). At the next dose level, ≥500 µg/plate, bacteriotoxicity was reported with S9 fraction only.

To confirm these findings, TA 100 was re-tested without S9 using dose ranges of 0-80 and 0-40 µg/plate. The three remaining strains were also re-tested at 0-20 µg/plate without S9. No increase in the number of revertants was reported with TA 1535, TA 1537 and TA 98. In re-tests with TA 100, an increase in revertants was reported at 0-80 µg/plate (1.9 - 2.7x) and 0-40 µg/plate (1.8 - 2x). No increase in bacteriotoxicity to TA 100 was observed.

As the positive control, endoxan (cyclophosphamide) which requires metabolic activation was used in the above experiments (without S9). No response was observed in the positive control plates. This does not allow the responsiveness of the strain to be determined and therefore, the study is considered to be flawed. The criteria for a positive result were fulfilled. In this study dichlofluanid is positive with and without S9. [Unpublished, 1979]

In a further Ames test, concentrations in the range of 0-800 µg/plate were initially used both with and without S9. The purity of the dichlofluanid was 92.2 %. The positive controls used were endoxan for strains TA 1535 and TA 100, and trypflavine for TA 1537 and TA 98; in addition 2-aminoanthracene was used with all strains. In the absence of S9 no increase in revertants was reported and bacterial growth was completely abolished at 50 µg/plate in all strains. In the presence of S9, TA 1535 was negative. TA 100 was positive at 100 and 200 µg/plate (both 1.8x), with a 33 and 45 % decrease in growth respectively. TA 1537 was positive at 200 µg/plate only (2.3x), with a 3 % decrease in growth. TA 98 was positive, with a dose-dependent increase in revertants from 25 (2.1x) to 200 µg/plate (3.7x), with a 27 % decrease in growth at 200 µg/plate. In the presence of S9, growth was completely abolished at 400 µg/plate.

To confirm these findings repeat experiments were carried out with all strains at 0-48 µg/plate without S9. In addition, TA 100, 98 and 1537 were tested at 0-304 µg/plate with S9.

No increase in the number of revertants was reported with TA 100, 1535 and 1537 without S9, and with TA 1535 and 1537 with S9. A dose-dependent increase in the number of revertants from 3 (2x) to 24 μg/plate (3.9x) with TA 98 was reported in the absence of S9. No bacteriotoxicity was observed at these doses. In the presence of S9 a dose-related increase in revertants was reported (1.6x, 2x and 2.2x at 76, 152 and 304 μg/plate respectively) and in TA 100 with a 76 % decrease in growth at 304 μg/plate. A dose-dependent increase in the number of revertants, from 76 (2.3x) to 304 μg/plate (2.8x), was also reported in TA 98. With this strain the observed bacteriotoxicity was negligible; 13 % at 304 μg/plate.

As the positive controls gave appropriate responses, dichlofluanid was considered mutagenic under the conditions of this study. [Unpublished, 1984]

A published study using *S. typhimurium* strains TA 102 and 104, with and without S9 fraction, is available. Bacteria were exposed to dichlofluanid (99 % purity) at 0-2.5 μg/plate. The positive controls were 2-aminoanthracene (with S9) and methylglioxal (without S9) with TA 102. Methylmethane sulphonate replaced methylglioxal with TA 104. No increase in the number of revertants was reported with dichlofluanid, and as the positive controls performed as expected. Dichlofluanid was not mutagenic in this study. No further information was provided. [Barrueco and de la Peña, 1988]

A poorly reported reversion assay is available using *Escherichia coli* strain WP2 hcr and *S. typhimurium* strains TA 1535, 1537, 1538, 98 and 100. Assays were performed both with and without aroclor induced rat liver S9. The positive controls used were 2-aminoanthracene, β–propiolactone, 9-aminoacridine and 2-nitrofluorene. Dichlofluanid caused an increase in the number of revertants with *S. typhimurium* strain TA 98 (3-4x) both with and without S9, from 50 μg/plate. Dichlofluanid also caused an increase in the number of revertants with TA 100 (2x) at 50 μg/plate (-S9) and 100 μg/plate (1.7 x with S9).

Increases in the number of *E. coli* revertants were also reported, without (6.4x at 50 μg/plate) and with S9 (2x and 2.7x at 50 and 100 μg/plate). Insufficient information was provided to determine the extent of bacteriotoxicity of dichlofluanid in the above assays.

As the positive controls responded appropriately in the presence of an S9 fraction (10-100x increase in revertants). Dichlofluanid is considered mutagenic under the conditions of the study. [Unpublished, 1978]

In a mutagenesis screen, dichlofluanid (unspecified purity) was reported to give a positive response in a recombination assay using *E. coli* strain WP2 hcr, and in a reversion test with *S. typhimurium* (TA 100 and 98 strains). Dichlofluanid was tested up to 5000 μg/plate. No further information was provided. [Unpublished, 1983]

A recombination assay is available using *Bacillus subtilis* strains H-17 (with recombination repair system) and M-45 (without recombination repair system). Assays were performed both with and without an aroclor induced rat liver S9 fraction. The controls were kanamycin (positive) and mitomycin C (negative). Test solutions of dichlofluanid (98.9 % purity) were

prepared in DMSO; 20 µl of each was allowed to soak into a 10 mm filter paper disc (0-20 µg/disc). The disc was then placed at one end of a single streak of cells and incubated for 24 h. The difference in length of inhibitory zones between competent and deficient strains was the basis of the scoring system. The maximum difference reported with dichlofluanid was at 20 µg/disc (3 mm). As the positive controls gave appropriate responses, kanamycin (1.5 mm) and mitomycin C (9 mm), dichlofluanid was considered to be weakly mutagenic by the study authors. [Unpublished, 1978]

In a further mutagenesis assay using *B. subtilis* strains M45 and H17, dichlofluanid (unspecified purity) also gave a positive response in the absence of a metabolic activation system at a concentration of 1 µg/disc. No further information was provided. [Unpublished, 1975]

3.3.3.2 *In Vitro* Studies With Mammalian Cells

Dichlofluanid (89-90.4 % purity) was tested for potential to cause gene mutations at the HGPRT locus in cultured Chinese Hamster Ovary cells (CHO), with and without metabolic activation. This study was conducted to GLP and was essentially Annex V compliant. The positive controls were ethylmethane sulphonate in the non-activation assay and 3-methylcholanthrene in the activation assay. Negative controls used were untreated cells and vehicle-only treated cells. A response was considered positive if a reproducible, dose-dependent doubling (preferably 3 doses) of the mutation frequency was observed.

Following a range-finding study, 7 concentrations from 0-95 µg ml^{-1} were selected without S9 and 9 concentrations from 0-12 µg ml^{-1} with S9.

In the range-finding test a relative survival of 52 % was noted at 0.5 µg ml^{-1} in the non-activation assay, and of 50 % in the activation assay at 1.56 µg ml^{-1}. Unless stated otherwise, relative survival is compared to solvent controls.

Three trials were conducted for the main study. In the non-activation test the relative survival was decreased, although not dose dependently (67-73 % maximum). There were no increases in the number of mutant colonies. In the activation assay, in the first test, a statistically significant increase in the mutation frequency was reported at 6 µg ml^{-1} and above; (1-5x). The relative survival was decreased by 40 % and 75 % at 6 and 12 µg ml^{-1} respectively. The mutagenic effects were not dose dependent. These findings were not confirmed in the repeat test. No precipitation was reported throughout the study.

As the increase in the number of mutant colonies was not confirmed, and the positive controls responded appropriately, then dichlofluanid was not considered to be mutagenic to the HGPRT gene in CHO cells in this study. [Unpublished, 1988(d)]

Dichlofluanid (89-90.4 % purity) was tested for the potential to cause gene mutations at the HGPRT locus in V79 cells, with and without metabolic activation. This study was conducted to GLP and was essentially Annex V compliant. The positive controls used were ethylmethane sulphonate in the non-activation test and 3-methylcholanthrene in the activation

test. Negative controls used were untreated and vehicle-only treated cells. A response was considered positive if a reproducible, dose-dependent doubling (preferably 3 doses) of the mutation frequency was observed. Following a range-finding experiment, 6 concentrations from 0-0.35 μg ml^{-1} without S9 and 0-20 μg ml^{-1} with S9 were chosen.

In the range-finding test, 100 % survival was reported at 0.2 μg ml^{-1} and 1% at 0.4 μg ml^{-1} in the non-activation assay. In the activation assay, 75 % survival was reported at 12.5 μg ml^{-1} and 1 % at 25 μg ml^{-1}.

Duplicate tests were conducted for the main study. In the non-activation test, the relative survival was variable (60-82 % at 0.35 μg ml^{-1}). No increase in the number of mutant colonies was reported. In the activation assay the relative survival was decreased, in both test, at the highest concentration (53 and 88 % at 20 μg ml^{-1}). No increase in the number of mutant colonies was reported. No precipitation was reported in this study. As the positive controls gave appropriate responses, dichlofluanid does not cause mutations in the HGPRT gene in V79 cells. [Unpublished, 1988]

Dichlofluanid (89 % purity) was tested for the potential to cause gene mutations at the TK locus in mouse lymphoma cells, with or without metabolic activation. This study was conducted to GLP and was essentially Annex V compliant. The positive controls used were ethylmethane sulphonate in the non-activation assay and 3-methylcholanthrene in the activation assay. Negative controls used were untreated cells and vehicle-only treated cells. Dichlofluanid was cytotoxic at 0.5 μg ml^{-1} without S9 and at 7.81 μg ml^{-1} with S9. No further information was provided regarding the range-finding test. Cells were treated with concentrations in the range of 0.10-1.2 μg ml^{-1} without S9 and 0-12 or 16 μg ml^{-1} with S9.

In duplicate non-activation tests the relative survival was in the range of 6%-80 %. The mutation frequency was increased at 1.2 μg ml^{-1}; in the first test by 3.3x (6.7 % relative survival) and 2.2x (16 % relative survival) in the repeat test. In the first activation test (0-12 μg ml^{-1}) a 3x increase in the mutation rate was reported (19 % relative survival) at 12 μg ml^{-1}. In a repeat test carried out with 0-16 μg ml^{-1}, an increase in mutation rate was reported from 4 μg ml^{-1} (62 % relative survival), and 3x greater than the solvent control rate at 16 μg ml^{-1} (18 % relative survival).

As the positive controls gave appropriate responses, dichlofluanid was found to be mutagenic both with and without an S9 fraction. [Unpublished, 1985(a)]

The ability of dichlofluanid to cause chromosome aberrations was investigated using human lymphocytes. This study was conducted to GLP and was essentially Annex V compliant. The positive controls used were mitomycin C and cyclophosphamide. A vehicle-containing negative control was also used. The lymphocytes were obtained from healthy volunteers (one/sex/group). The concentrations used were selected following a range-finding experiment and were in the range 0-10 μg ml^{-1}.

In the range-finding test, the percentage of mitoses were stated to be reduced in comparison to controls. However no further information was provided. In the main test the mitotic rate

was reduced in all treatment groups, 50 % and 44 % of control at 3 μg ml^{-1}, without and with an S9 fraction respectively (10 and 14 % at 10 μg ml^{-1}).

Forty-eight hours after lymphocyte preparation, the test substance (and S9 fraction if needed) was added, 2.5 h later the S9 fraction was washed off. After 69 h colcemid was added to arrest the cells which were prepared for recording at 72 h. Two hundred metaphases per dose were examined for clastogenicity. In the absence of S9 a statistically significant increase in metaphase aberrations was reported at 3 μg ml^{-1} (7.5 % including and 6 % excluding gaps) and 10 μg ml^{-1} (15.5 % including and 15 % excluding gaps). The positive control, mitomycin C, did not cause a response. In the presence of S9 a statistically significant increase in metaphase aberrations (14 % including and 13 % excluding gaps) was reported at 10 μg ml^{-1} only. The aberrations caused by dichlofluanid treatment were predominately breaks. The positive control, cyclophosphamide, gave appropriate responses.

Dichlofluanid was positive for chromosome aberrations in the presence of S9 at a cytotoxic dose. However in the absence of S9, the significance of the increase is unclear as the positive control failed to respond. [Unpublished, 1986(b)]

3.3.3.3 *In Vivo* Studies

A mouse bone marrow micronucleus test is available (5/sex/group NMRI strain). This study essentially complies with Annex V guidelines. Animals were administered two doses of dichlofluanid (100 % purity) by gavage, 24 h apart, either at 1000 or 2000 mg kg^{-1} in a cremopohor emulsion. The positive control used was endoxan. Individual animals were reported to have lost weight or were constipated. Animals were sacrificed for bone marrow examination 6 h after the final dose. One thousand polychromatic erythrocytes were examined per animal. An apparent depression of bone marrow activity was reported, as the NCE:PCE ratio was found to increase dose dependently. The NCE:PCE ratios in the first test were 596.3, 753.2 and 1089 at 0, 1000 and 2000 mg kg^{-1} respectively. In the second test the ratios were 1072.9 and 679.6 at 0 and 2000 mg kg^{-1} respectively. No increases in the number of micronuclei were reported in any of the groups receiving dichlofluanid.

A repeat experiment to confirm this finding was carried out using either 0 or 2000 mg kg^{-1} with the same protocol. No positive results were reported and the bone marrow depression could not be confirmed.

As the positive control gave appropriate responses, dichlofluanid did not cause an increase in the number of micronuclei under the conditions of the study. [Unpublished, 1978(d)]

In a cytogenetic study carried out in the Chinese hamster, animals were administered two doses of dichlofluanid; either 0, 250 or 500 mg kg^{-1} (100 % purity) by gavage, 24 h apart. A dose of 1000 mg kg^{-1} was originally included, but was withdrawn due to excessive toxicity; no further information was provided. The positive control, adriabalastin, was administered at 5 mg kg^{-1} i.p.; negative control animals received vehicle only. Forty-three hours after the final dose animals received colchicine 4 mg kg^{-1} i.p., and at 48 h animals were sacrificed. No signs of toxicity were reported, although one animal died in the top-dose group (not treatment-related). One hundred spermatagonia metaphases were prepared and examined. No

treatment-related increases in chromosome damage were reported. No further information was provided.

As the positive controls gave appropriate responses then, dichlofluanid did not damage spermatagonia chromosomes under the conditions of the study. [Unpublished, 1979(b)]

A single unscheduled DNA synthesis (UDS) study was carried out using male rats (Wistar 3/group). This study was carried out to GLP and EPA standards and was also Annex V compliant. Animals were administered either 0, 170, 500, 1500, or 4500 mg kg^{-1} dichlofluanid (92 % purity) by gavage in cremophor EL (0.5 % aqueous suspension). Two exposure periods were used; 2 and 16 h. An additional dose was included (100 mg kg^{-1}) in the 16-h exposure group. Two positive controls were used; methyl methane sulphonate (2-h exposure) and 2-acetamidofluorene (16-h exposure). After the appropriate exposure period, animals were sacrificed and hepatocytes isolated in order to determine the extent of UDS. Necropsy of the animals in the top-dose group revealed congested lungs and excessive gas in the intestine and stomach. No evidence of hepatotoxicity was reported. No evidence of UDS was found at either time-point with the dose range used. The percentage of cells in repair can be seen in Table 3.6.

Table 3.6 : Percentage Of Cells In Repair

treatment	**2 h**	**16 h**
control	0.5±0.4	0.9±0.8
450 mg kg^{-1} dichlofluanid	1.1±1.4	0.7±0.7
positive control	42.5±5	42.5±5

As positive controls gave appropriate responses, it was concluded that dichlofluanid did not cause UDS under the conditions of the study. [Unpublished, 1988(f)]

Two *in vivo* Chinese hamster bone marrow chromosome aberration studies are available (inbred strain). Both were essentially Annex V compliant and carried out to GLP standards. However, the 1988 study used 5 instead of 10 animals and in the 1989 study; no harvest was carried out at 24h. Dichlofluanid was prepared in cremophor EL (0.5 % aqueous suspension). The positive control, cylophosphamide 30 mg kg^{-1}, was prepared in distilled water. The same dosing volume was used in both studies; 10 ml kg^{-1} .

In the first study animals were administered dichlofluanid (91.4 % purity) by gavage at either 0, 1000, 2000 or 4000 mg kg^{-1}; control and low dose (6 group/sex) and middle and high dose (10 group/sex). One female died at the middle dose, and 5 males and 3 females at the top dose. The positive control animals were sacrificed after 24 h and all others after 42 h. Two hours prior to sacrifice all animals received an i.p. injection of colcemid (2 mg kg^{-1}) to arrest cells in metaphase. The prepared spreads (50/animal) were examined for a variety of breaks. No increase in the numbers of aberrations was reported. The mitotic index was decreased in all treatment groups and positive controls. See Table 3.7.

Table 3.7 : Mitotic Index In Study (%)

treatment	mitotic index (%)
positive control	2.3
control	4.5
1000 mg kg^{-1}	2.6
2000 mg kg^{-1}	2.7
4000 mg kg^{-1}	2.8

It was concluded that as the positive control performed as expected; dichlofluanid did not cause chromosomal aberrations under the conditions used. No further information was provided. [Unpublished, 1989(c)]

In the second study animals (5 group/sex/time point) received a single dose of either 0 or 10,000 mg kg^{-1} dichlofluanid (90.4 % purity) by gavage. This dose was chosen after a range-finding study using either 0, 1000, 2500 or 5000 mg kg^{-1}. No deaths were reported, however above 1000 mg kg^{-1} animals exhibited apathy, ruffled fur and weight loss. No evidence was provided concerning bone marrow toxicity. The positive control used throughout this study was cyclophosphamide.

The study was divided into two, depending on time of sacrifice. Two hours prior to sacrifice all animals received an i.p. injection of colcemid (3.3 mg kg^{-1}) to arrest cells in metaphase. In the first experiment animals were killed at 6, 24 and 48 h. All control animals were sacrificed at 24 h. In the second experiment, animals receiving dichlofluanid were killed at 24, 32 and 40 h. One hundred metaphase spreads per animal were examined for aberrations.

At the administered dose 9/39 animals died in the first study and 10/40 in the second. Signs of toxicity reported in the first experiment were bloody noses, somnolence, prone position and ruffled fur. In the second study apathy, reduced motility, feeble reflexes, distended abdomen, spastic gait, prone position, convulsions, accelerated breathing, spanopnea, gummy eyes and closed palpebral fissures were reported.

In the first experiment a statistically significant increase in the number of aberrations was reported at 48 h (3.5 %, including gaps). Statistically significant increases in the number of aberrations were also reported at 24 h (1.77 %) and 48 h (2.34 %), excluding gaps. In the second experiment statistically significant increases in the number of aberrations were reported at 32 (4.3 %) and 40 h (5 %), including gaps. A significant increase was reported at 40 h only (2.5 %), excluding gaps. The reported increases were in comparison to negative controls.

As the positive control gave appropriate responses, dichlofluanid was considered capable of chromosome aberrations under the conditions of this study. [Unpublished, 1988(a)]

In a mouse dominant lethal test, males (Bor:NMRI SPF han strain) were administered a single dose of either 0, 2500 or 5000 mg kg^{-1} dichlofluanid (91.7 % purity) in a 0.5 % cremophor emulsion by gavage. Sufficient animals were treated to cover intercurrent deaths. This study was carried out to GLP; Annex V compliance depends on the alleged positive

control data being supplied by the Applicant. Six males died in the middle- and 13 in the top-dose group. Signs of toxicity reported were apathy, closed eyelids and feeble reflexes, which resolved after 72 h. Treated males, 50 per group, were mated with untreated females; 600 in the control and 600 or 579 in the treatment groups. Each male was sequentially mated with 12 untreated females over a 48 d period. Females remained with a male for 4 d before being removed and replaced. Females were sacrificed 12 d after the end of the relevant mating period.

No negative effects on fertilisation or on pre/post implantation losses were reported. Therefore, dichlofluanid does not damage spermatogonia or spermatocytes under the conditions of this study. [Unpublished, 1986(d)]

A mouse spot test was carried out with a cross between C57BL/6j females and T males. Following a range-finding study, animals received a single dose of either 0, 400, 800 or 1600 mg kg^{-1} dichlofluanid (90 % purity) by gavage in 0.5 % cremophor emulsion. This was not carried out to GLP but was essentially Annex V compliant. Sufficient females were treated on day 10 of gestation to provide 300 F1 progeny. The positive control substance, ethylnitrosourea, was administered i.p.. Deaths were reported all dose levels; 12/198 (400 mg kg^{-1}), 13/196 (800 mg kg^{-1}) and 26/340 (1600 mg kg^{-1}) respectively. The signs of toxicity reported were apathy, reduced motility, emaciation, prone position, slow breathing, breathing difficulties, sunken flanks, rough fur, silted eyes, gummed eyelids and diarrhoea. These signs of toxicity were apparent immediately post dosing, but resolved during the study. Pups were examined for coat spots at 12-16, 20-25 and 30-50 d post partum.

No treatment-related increases in the numbers of spots were reported. As the positive control gave appropriate responses, dichlofluanid does not cause fetal somatic mutations under the conditions of the study. [Unpublished, 1988(b)]

3.3.3.4 Studies Conducted To Non-Standard Protocols

A sister chromatid exchange (SCE) study is available (Chinese hamster 5/sex/group). Animals received dichlofluanid (100 % purity) at 1000 or 2000 mg kg^{-1} by gavage in 0.5 % cremophor emulsion. The positive control was 20 mg kg^{-1} endoxan by gavage; the negative controls received vehicle only. At 2 h pre-dose, animals received 50 mg bromodeoxy uridine s.c., and 22 h post dose colchicine 4 mg kg^{-1} i.p.. Animals were sacrificed 24 h post dose. No deaths or signs of toxicity were reported. Bone marrow smears were prepared and examined for SCE's. No increases in the rate of sister chromatid exchange were reported.

As the positive control gave appropriate responses, dichlofluanid did not cause an increase in the number of sister chromatid exchanges under the conditions of the study. [Unpublished, 1980(c)]

Dichlofluanid (unspecified purity) was given by gavage at 200 mg kg^{-1} in DMSO (4 or 5 animals). Autoradiography of ^{3}H-thymidine labeled samples was used to measure testicular DNA synthesis. Dichlofluanid was reported to cause a statistically significant decrease in the incorporation of ^{3}H-thymidine into DNA and was therefore positive. [Unpublished, 1977]

The genotoxicity of dichlofluanid was investigated in the following 7 assay systems. A commercially available formulation containing 50 % dichlofluanid was used to prepare the solutions for each assay (20 mmol dichlofluanid in DMSO). The tests were : a DNA synthesis inhibition test (DIT); a DNA viscosity test; the *umu*-test, with induction of β–galactosidase as the end point; a fluorescence assay of DNA unwinding (FADU); a fish test using *Leuciscus idus melanotus,* hepatic DNA samples were prepared using alkaline filter elution with the endpoint of DNA damage; EM visualisation of DNA strand breaks; and a ^{32}P post-labeling test. Metabolically activated benzo(a)pyrene and nitroquinoline-N-oxide were used as positive controls for the DIT, alkaline viscometery, *umu*-test and the FADU test. Benzo(a)pyrene was also used as a positive control in the ^{32}P post-labeling test. None of the tests were carried out in the presence of a metabolic activation system. Dichlofluanid was found to be positive in each of these tests. As the positive controls responded appropriately, this was interpreted as dichlofluanid being potentially genotoxic. A summary of the methods used was provided. Dichlofluanid (unspecified purity) was reported to give negative results in an *E. coli* liquid-holding assay to detect forward mutations to streptomycin resistance and a spot test to detect forward mutations to 5-methyl tryptophan resistance. [Unpublished, 1974]

A negative result was also reported with dichlofluanid (unspecified purity) in a spot test to detect reverse mutations in *Serratia marcescens* and reverse mutations to galactose prototrophy in gal-*E. coli*. No further information was provided. [Unpublished, 1974]

In a published paper the ability of dichlofluanid to cause genetic damage was investigated in *Saccharomyces cerevisiae*. A suspension of dichlofluanid (unspecified purity), of unknown concentration, was prepared at pH 3.0, 6.0 and 7.5. This suspension was incubated with stationary phase cells at 25 ^{o}C for "several hours" in the absence of a metabolic activation system. The cell numbers were adjusted to 10^7 ml^{-1} and plated on selective media. As dichlofluanid caused an increase in cytoplasmic mutation (50 ppm) and mitotic conversion, this was interpreted by the study authors as a weakly positive response. No positive control was included. [Unpublished, 1970]

3.3.3.5 Summary Of Genotoxicity

Dichlofluanid has been thoroughly tested for genotoxic potential in a range of *in vitro* and *in vivo* studies. Dichlofluanid was found to be a bacterial point mutagen, and cause mutations at the TK locus in eukayotic cells. A positive result was obtained in an *in vitro* cytogenetics assay. However, when tested *in vivo*, negative results were obtained in a mouse micronucleus, liver UDS, and one somatic cell chromosome aberration test. Further *in vivo* testing in a mouse spot test, a sperm cell chromosomal aberration cell test and a rodent dominant lethal test were all clearly negative. The available data indicate that dichlofluanid was not an *in vivo* somatic cell or germ cell mutagen. Overall dichlofluanid is unlikely to pose a genotoxicity hazard to man.

3.3.4 CHRONIC TOXICITY

3.3.4.1 Rat Two-Year Chronic Toxicity

A poorly reported 2-year study not conducted to GLP is available. Wistar rats (SPF 40/group/sex, control 80/sex) received dietary administration of either 0, 150, 1500 or 4500 ppm dichlofluanid (90 % purity). This was approximately equivalent to 6.9, 23.3, 69.1 or 218.6 mg kg^{-1} d^{-1} and 8.4, 29.4, 85.5 or 279.7 mg kg^{-1} d^{-1} for males and females respectively. Interim blood samples were taken at 4 and 24 weeks. Intercurrent deaths were reported in all groups but were not considered to be treatment-related. See Table 3.8. The cause of death was given as hypertony or atony of the intestine; a common cause of death among SPF rats.

Table 3.8 : Intercurrent Deaths Recorded During The Study

dose (ppm)	males	females
0	23/80	28/80
150	13/40	11/40
500	11/40	5/40
1500	10/40	7/40
4500	9/40	8/40

Food consumption was unaffected. However, body weights of males in the top-dose group were significantly decreased at 12 months, and in both sexes at study termination (8 % males and 10 % females respectively).

At 24 months there were no treatment-related effects on kidney function, liver macro- or micropathology, clinical chemistry or haematology. At necropsy some changes were reported in absolute and relative organ weights. No observations concerning neoplasms were made. The absolute liver weights in males were increased at all doses, but were only statistically significant at the middle dose (14 %). An increase in absolute testes weight was observed at all doses (10-12 %, 150-4500 ppm); these increases were not statistically significant. There was a significant decrease in absolute thyroid weight in the top-dose group of both sexes (20 % and 28 % males and females respectively).

At 24 months relative liver weights were significantly increased in males (17.2 %) and females (4.2 %) at the top dose, which in males was dose-related. There was also a significant increase in the relative kidney weight (12.5 %) of females in the top-dose group only. A non-significant increase in testes weights was apparent; 20 % at the top dose. These changes could reflect the decrease in body weight reported at the top dose.

The NOEL was 1500 ppm (estimated to be 218 mg kg^{-1} d^{-1} and 279 mg kg^{-1} d^{-1} for males and females respectively) based on liver and kidney enlargement and slight testicular effects observed at 4500 ppm. The Applicant also considered this to be the NOEL. [Unpublished, 1968]

3.3.4.2 Mouse Two-Year Chronic Toxicity

A 2-year study is available. No evidence was supplied to indicate compliance with either GLP or contemporary guidelines. SPF mice, CF1 strain (50/group/sex), received dietary administration of either 0, 200, 1000 or 5000 ppm dichlofluanid (90 % purity). This was calculated to be equivalent to 49, 252 or 1317 mg kg^{-1} d^{-1} and 57, 273 or 1638 mg kg^{-1} d^{-1} for males and females respectively. In addition, two satellite groups (5 animals/sex/dose) were scheduled for interim kills at 6 and 12 months. The mortality rate was unaffected by the treatment regime (10 % control and 16 % at 5000 ppm males, and 8 % control and 20 % at 5000 ppm females). There were no overt signs of toxicity in any treatment group throughout the study period. The body weights of males were reduced at the interim kills but were indistinguishable from control animals at study termination. In females however, body weights were comparable to controls at the interim kills, but at study termination a statistically significant decrease was reported at the top dose (15 %).

The haematological findings were not considered of toxicological significance.

The clinical chemistry data at six months indicated significant decrease in the serum protein concentration of males (11 %) in the top-dose group. At 12 months there were variations in serum transaminase levels which were not considered to be of toxicological significance. At 24 months the serum protein levels of males in the low- and top-dose groups were significantly raised (13 and 12 % respectively). The ALP levels were raised in all treated males at study termination and reached statistical significance in the middle- and top-dose groups (14 and 12 % respectively). In females at study termination, the levels of ALP (77 %), bilirubin (50 %) and cholesterol (20 %) were elevated at the top dose.

There was a significant decrease in the serum creatanine levels in males of the top-dose group after 6 months (27 %). Females at study termination were found to have increased serum urea levels at all doses, which reached statistical significance in the top-dose group only (20 %).

Necropsy of intercurrent deaths revealed hardened crania and hard nodes on the femurs and vertebra of females at the top dose. There were no dose responses or chronological relationships in either the absolute or relative organ weights. With the exception of increased skeletal malformations at study termination in females at the top dose (7 individuals), described as medullated bone proliferation, there were no pathological or histopathological observations attributable to the treatment.

An increase in the number of lung adenocarcinomas was found in females (0, 1, 3 and 3 at 0, 200, 1000 and 5000 ppm respectively). These are considered to be common tumours in mice (incidence of 5.5 % in B6C3F1 and 15 % in CD1 mice). No further treatment-related increases in tumour rate (benign or malignant) could be found. The NOAEL for non-neoplastic events should be 1000 ppm; equivalent to 252 and 273 mg kg^{-1} d^{-1} in males and females respectively. This is based on elevated ALP levels in both sexes and elevated markers of biliary and kidney function reported at 5000 ppm. The NOEL for neoplasms is 5000 ppm; equivalent to 1317 and 1638 mg kg^{-1} d^{-1} for males and females respectively. There was no dose response, and the available data (different strains) suggests the observed tumour incidence in this study is not unusual. [Unpublished, 1982(a)]

3.3.4.3 Summary Of Chronic Toxicity

The summary of the chronic toxicity studies has been combined with the repeated-dose toxicity summary. See section 3.3.2.3.

3.3.5 COMBINED CHRONIC TOXICITY AND CARCINOGENICITY

3.3.5.1 Rat Two-Year Combined Chronic Toxicity And Carcinogenicity Study

A 2-year combined chronic toxicity and carcinogenicity study is available. This study was carried out to EPA guidelines and was both GLP and Annex V compliant. Wistar rats (BOR:WISW strain, 50 group/sex, plus 10/sex group interim kill) received dietary administration of either 0, 180, 900 or 4500 ppm dichlofluanid (89-93 % purity). This was equivalent to 9.4, 54.4 or 301.3 mg kg^{-1} d^{-1} and 13.5, 73.1 or 420.7 mg kg^{-1} d^{-1} for males and females respectively. Those animals scheduled as interim kills were sacrificed after 53 weeks and study termination was scheduled for 106 weeks. Interim blood samples were taken at weeks 26, 53 and 79 for clinical chemistry and haematology. Urinalysis was also carried out at these time points.

Deaths were reported in both the satellite and main groups. In the main study the terminal mortalities were 12 %, 18 %, 30 % and 16 % at 0, 180, 900 and 4500 ppm in males and 26 %, 26 %, 22 % and 20 % at 0, 180, 900 and 4500 ppm in females. In the satellite group 2 males died in the top-dose group. These deaths were not considered to be treatment-related.

In the top-dose group after 8 weeks, reddish/brown flecks were reported around the snout and significantly increased incisor growth was reported after 20 weeks (males and females). Unspecified nasal skin changes were also noted; no further information was provided. All other observations, body surfaces, orifices, behaviour, posture, respiration, opthalmoscopy and excretory products were unaffected by the treatment regime. The body weights of both males and females were significantly decreased at the top dose throughout the study period (9.2 % and 15 % males and females respectively at study termination). A 6.7 % body weight decrease in males at the middle dose was also noted. Food consumption was unaffected by the treatment regime.

No toxicologically significant haematological effects were reported. Clinical chemistry analysis showed that serum albumin levels in females were in general decreased throughout the study. At study termination there was a dose-dependent decrease, which was statistically significant at the intermediate (32 %) and top doses (30.5). This decrease was mirrored by a statistically significant decrease in total protein levels at each dose (66.7 % at 4500 ppm). Serum calcium concentrations were decreased in females at the intermediate and top doses (7 % at 4500 ppm), although all values were within ±2SD of the historical control mean value.

A significant increase in the urine volume of males in the top-dose group was reported at week 53 (125 %). At weeks 79 (125 % at 4500 ppm) and 104 (100 % at 4500 ppm) this increase appeared to be treatment related. In females urine volumes were increased at the top

dose from week 55* onwards; 75 %, 28 % and 42 % at 55, 79 and 105 weeks respectively (*statistically significant).

The fluoride content of bones and teeth exhibited a dose-dependent significant increase in all treatment groups of both sexes at 53 weeks and study termination. See Table 3.9. The calculated fluoride intakes were 0.5, 3.1 and 17.1 mg kg^{-1} d^{-1} (males) and 0.8, 4.2 and 23.9 mg kg^{-1} d^{-1} (females) at 180, 900 and 4500 ppm respectively.

Table 3.9 : Fluoride Content Of Bones And Teeth At Study Termination

males				
site	**0 ppm**	**180 ppm**	**900 ppm**	**4500 ppm**
teeth*	0.12	0.27	1.17	3.92
bones*	0.43	0.97	2.91	13
females				
site	**0 ppm**	**180 ppm**	**900 ppm**	**4500 ppm**
teeth*	0.11	0.32	1.12	5.36
bones*	0.66	1.44	3.53	11.46

* mg fluoride/g ash

The pathology report on the interim kills revealed "a whitish hardening" of the cranium (still present at study termination). At the interim kill, the absolute heart weight was significantly decreased in both sexes at the top dose only (18 % and 14 % males and females respectively). The increases in relative liver weight in both sexes, and relative kidney and brain weight in females, reported at 4500 ppm reflected the previously noted decreases in body weight.

In males at study termination the absolute organ weights of the kidney (5 %) and testes (5.6 %) were significantly increased at the top dose. At study termination the relative testes weight was increased in the top-dose group (18 %). The reported increases in relative organ weights reflected the decreases in body weights noted earlier.

Histopathology of the satellite groups revealed a dose-dependent increase in cranial osteosclerosis in females, achieving statistical significance in the middle (8/10) and top-dose groups (9/10) when compared to control animals (1/10). In males however a significant increase in cranial osteosclerosis was only found in the low- (7/10) and top-dose groups (9/10); control (1/10). An increase in cranial lamellar growth patterns was also reported in males at the top dose (7/10, and controls 0/10). The forestomachs of males from the top-dose group were found to have a higher incidence of hyperkeratosis and acanthosis (0/10 and 5/10 controls and 4500 ppm respectively). The thyroids of males exhibited follicular cell hypertrophy; 0/50 (control), 2/10 (180 ppm), 1/10 (900 ppm) and 3/10 (4500 ppm).

Histopathology of the main groups (24 months) revealed a significant increase in hyperkeratosis and acanthosis of the forestomach in males (2/49 and 21/49 controls and 4500 ppm respectively) and females (0/49 and 12/49 controls and 4500 ppm respectively). A significant increase in the incidence of cranial osteosclerosis was apparent in males of all treatment groups and in females at the top dose. These data also suggest an increase in severity with increasing dose. See Table 3.10.

Table 3.10 : Incidence And Severity Of Cranial Osteosclerosis

	males (dose ppm)				females (dose ppm)			
grade	**0**	**180**	**900**	**4500**	**0**	**180**	**900**	**4500**
minimal	0	6	4	0	12	1	1	1
slight	11	14	24	4	0	7	13	8
moderate	0	11	6	18	0	10	2	22
marked	0	3	0	26	0	2	0	9
severe	0	0	0	1	0	0	0	6
total	11	34*	34*	49*	12	20	16	46*

* statistically significant

A significant decrease in sternal chondrodystrophy reported in females of the top-dose group. A significant increase in cranial lamellar growth patterns was reported in both sexes at the top dose (45/49 and 37/50 males and females respectively) compared to the controls (1/49 and 0/50 males and females respectively). In the thyroid gland a statistically significant increase in the lesion described as a focal follicular growth anomaly was established in both sexes at the top dose. In males the following data were presented : 1/50 (controls); 1/50 (180 ppm); 2/50 (900 ppm); and 3/49 (4500 ppm) and in females the data were : 0/50 (controls); 0/50 (180 ppm); 2/50 (900 ppm); and 4/49 (4500 ppm).

No treatment-related neoplastic alterations were reported in the satellite group. Tumours found were a malignant lymphoma (male 4500 ppm), a malignant fibrosarcoma of the skin (male 4500 ppm) and a benign pituitary adenoma (female 180 ppm). In the main group, treatment-related tumours of the thyroid were noted. See Table 3.11. The incidence of thyroid adenomas in supplied historical control data was 0.7 % and 1.2 % in females and males respectively. No information was available for carcinomas. These data indicate that the increased incidence of thyroid tumours reported is likely to be treatment related.

Table 3.11 : Showing The Distribution Of Thyroid Tumours

	males				females			
dose level (ppm)	**0**	**180**	**900**	**4500**	**0**	**180**	**900**	**4500**
follicular cell adenoma	1	0	0	5*	0	0	0	4
follicular cell carcinoma	0	0	0	1	0	0	1	1

n* = 49, all others n = 50

Two females sacrificed *in extremis* were found to have thyroid follicular cell carcinoma; one animal from each of the middle- and top-dose groups. No statistically significant neoplastic events were reported. However, a significant trend in the distribution of thyroid adenomas was found in both sexes.

A NOEL could not be set due to the effects of excess fluoride and cranial osteosclerosis at the lowest dose. The NOEL for neoplasms could be set at 900 ppm (equivalent to 54.4 and 73.1 mg kg^{-1} d^{-1} for males and females respectively) due to the increase in thyroid tumours seen at the top dose. [Unpublished, 1993(b)]

3.3.5.2 Summary Of Combined Chronic Toxicity And Carcinogenicity

The summary of the chronic toxicity of this chronic/carcinogenicity study has been combined with the summary of the repeated-dose toxicity. See Section 3.3.2.3.

3.3.6 CARCINOGENICITY

3.3.6.1 Mouse Carcinogenicity Study

A 2-year OECD-compliant carcinogenicity study carried out to GLP is available. Animals (SPF mice $B63CF_1$ 60 sex/group) received dietary administration of either 0, 200, 1000 or 5000 ppm dichlofluanid (89-93 % purity); equivalent to 50.1, 273.9 or 1731.3 and 63.7, 337 or 1872.7 mg kg^{-1} d^{-1} in females and males respectively. The scheduled study termination was after 104 weeks, with an interim kill at 52 weeks; all animals were necropsied plus any dying or sacrificed intercurrently. Haematological and clinical chemistry determinations were carried out at weeks 51-53 and 104/5.

Deaths were reported in all groups during the study. There was however no treatment-related increase in mortalities. At study termination the following mortalities were reported : 10 % and 16 % in males and 8 % and 20 % in females, at 0 and 5000 ppm respectively. The body weight gain of animals at the top dose was significantly decreased throughout the study period (18 and 16.5 % male and females, terminal) and also in males of the middle-dose group from week 40 (6 % terminal). There were however no effects on food consumption.

The findings reported after 52 weeks were as follows. There was a significant increase in thrombocyte numbers (17 % and 38 %, males and females respectively) at the top dose. The MCHC was found to be elevated in females at the top dose only. Significant decreases were reported in erythrocyte numbers (4 % males) and haemoglobin concentration (9 % females) at the top dose. The levels of the alkaline phosphatase increased dose dependently and reached statistical significance in animals receiving ≥1000 ppm. These increases were outside the reference range (±2 SD) in males receiving 5000 ppm and females receiving ≥1000 ppm. AST levels were elevated in males receiving 5000 ppm (24 %) and were also outside the reference range (±2 SD). The serum electrolytes sodium (2 %), potassium (6.25 %) and chloride (2 %) were all elevated in females at the top dose. No relevant reference range was available.

There were effects on both the relative and absolute organ weights. The following absolute organ weights were reduced : brain (5 % males and 6 % females), spleen (14 % females), and kidney (18 % females) at the top dose. The following relative organ weights of males were increased at the top dose: lung (31 %); liver (16 %); spleen (40 %); and testes (15 %). While

in females the relative liver weight was increased (18 %) and kidney weight decreased (10 %). The following neoplasms were found : harderian adenomas, one each at 200 and 1000 ppm in females; and at the top dose, one interstitial adenoma was reported in males.

The findings reported at study termination were as follows. Leukocyte numbers were significantly increased in all treated males and in females receiving ≥1000 ppm. This increase was apparently dose dependent in males, with 71 % and 203 % increases being reported at 5000 ppm for males and females respectively. The supplied reference data for leukocytes indicated that although all values were greater than the mean, only the top-dose values were outside the reference range (±2 SD).

The following haematology values were outside the supplied reference range (±2 SD), with the exception of the MCHC. There was a significant increase in thrombocyte numbers at the top dose (77 % and 133 %, males and females respectively). The MCHC was found to be elevated in males and significantly decreased in females; (3.3 % males and 7 % females respectively) at the top dose. Erythrocyte numbers, haemoglobin and haematocrit were decreased in males at the middle and top dose (26, 22 and 24.4 % at the top dose). All decreases were statistically significant, with the exception of the 1000 ppm haemoglobin value. A decrease in haemoglobin concentration (16 %) was apparent in females at study termination but was not statistically significant. No information on reticulocyte numbers was provided.

The plasma levels of ALP were significantly increased in males at dose levels >200 ppm and in females at all dose levels. The data reported in females were all outside the range of reference values and in males at the top dose only (209 % and 294 % at 5000 ppm).

The serum levels of the transamimases (AST and ALT) were also elevated at study termination; at the top dose. This was statistically significant for AST in males. The AST levels were elevated by 67 % and 41.5 % in males and females respectively. The ALT levels were elevated by 69 % and 65.5 % in males and females respectively. The reported transaminase data were at the upper limit of the reference range provided (±2 SD). Albumin (12 % males and 18 % females) and total protein (17 % males and 16 % females) levels were all decreased at the top dose. The albumin and protein values were at the lower end of the supplied reference range. There were other sporadic instances of significant deviations, but these were not considered to be of toxicological significance.

Gross pathological examination noted areas of hair loss in males of both the middle- and top-dose groups. Duodenal dysplasia was reported in both sexes at the top dose (21/42 males and 19/40 females, controls 0) and gastric dysplasia in males only (10/42, controls 0). There were reports of femoral (19/40) and patellar hyperosteosis (4/40) in females in the top-dose group; hyperosteosis was not reported in the controls. Renal basophilic nephropathy was found in both sexes at the top dose (4/45 controls and 36/42 at 5000 ppm males) and (2/46 controls and 16/40 at 5000 ppm females). Pigmentation of mandibular lymph node macrophages was found to be increased in females at both the middle (8/46) and top doses (8/46) compared to the controls (1/46). In males increased pigmentation was apparent at the top dose only (0/4 controls and 4/42 at 5000 ppm). The number of oil red O staining hepatocytes was decreased in females at the top dose (27/42) compared to (40/45) in the controls. Increased thickness of the appositional bone of the cranial vault and nasal turbinates, also increased incidence of

tooth alveolitis, were reported in animals from the top-dose group. See Table 3.12. No increases in these findings were reported in other treatment groups.

Table 3.12 : Incidence Of Skull And Nasal Turbinate Thickness And Tooth Alveolitis Reported On Necropsy

	numbers			
	controls		5000 ppm	
pathology	**males**	**females**	**males**	**females**
skull thickness	0/45	0/46	39/42	40/40
turbinate thickness	0/45	0/46	23/42	35/40
tooth alveolitis	0/45	1/46	17/42	19/40

In those animals dying or sacrificed intercurrently, the following pathology was reported. There was an increase in basophilic nephropathy in males and increased thickness of the appositional bone, frontal bone and turbinates of females at the top dose. In addition, other unspecified bone changes and nephropathy were also reported.

The fluoride content of teeth and bone was found to be significantly increased at all doses and time points for both sexes. See Table 3.13.

Table 3.13 : Fluoride Content of Bones and Teeth (mg g^{-1} ash)

	teeth				bone			
	week 53		week 104		week 53		week 104	
dose (ppm)	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
0	0.4	0.4	0.2	0.3	1.1	0.9	1.1	1.2
200	0.7	0.6	0.7	0.8	2.4	1.5	3.6	2.9
1000	2.2	2.1	2.4	2.2	6	4.3	8.3	7.3
5000	10.9	9.9	15.7	13.8	17.4	14.5	18.7	17.5

There were effects on both the relative and absolute organ weights. In males, at the top dose, there were significant decreases in the absolute organ weights of the lung (20 %), brain (8 %) and testes (6 %). In females at the top dose there were significant decreases in the absolute organ weights of the brain (8 %), spleen (42 %) and kidney (18 %). The relative organ weights of the brain (13 %), liver (44 %), spleen (36 %) and testes (5 %) were increased in males at the top dose. In females the relative brain (7 %) and liver (47 %) weights were increased and the kidney (6 %) and ovary weights (28 %) were decreased. No incidences of toxicologically significant histopathology (liver included) were reported.

Table 3.14 : Summary Of Total Tumour Incidence Reported At Study Termination

	males				females			
dose (ppm)	**0**	**200**	**1000**	**5000**	**0**	**200**	**1000**	**5000**

animals with tumours	20/45	21/48	14/48	10/42	17/46	14/42	13/44	10/40
benign	13	16	9	7	9	5	7	8
malignant	5	3	4	3	8	8	3	1
benign/malignant	2	2	1	0	0	1	3	1
metastasising	1	1	1	0	7	7	3	0
total tumours	25	25	16	11	17	20	18	12

The most common sites of tumour formation were the lungs and liver, not uncommon in ageing mice. See Table 3.14 above. These two tumour types were within the supplied range of historical or concurrent control incidence. Sporadic instances of tumours at a variety of sites were also reported. There were however no dose- or treatment-related increases in numbers reported, therefore dichlofluanid was considered not to be carcinogenic under the conditions of the study.

It was not possible to establish a NOEL for non neoplastic effects. However a LOAEL of 200 ppm was established, equivalent to 50.1 and 63.7 mg kg^{-1} d^{-1} for males and females respectively based on reported increases in teeth and bone fluoride levels and elevated leukocyte numbers. A NOEL for neoplastic events was established at 5000 ppm for both sexes (equivalent to 1731.3 and 1872.7 mg kg^{-1} d^{-1} for males and females respectively). [Unpublished, 1993(d)]

3.3.6.2 Summary Of Carcinogenicity

The only carcinogenicity data relate to the oral route of exposure, with studies performed in the rat and mouse. No evidence of carcinogenic potential was observed in the mouse. However, in the rat dichlofluanid was found to cause an increase in the incidence of thyroid follicular cell tumours at the highest dose level of 300-420 mg kg^{-1} d^{-1}. These tumours occurred at a single site, were of late onset and generally benign pathology. Dichlofluanid has been thoroughly examined for genotoxic potential and the available data indicate that dichlofluanid was not an *in vivo* somatic cell or germ cell mutagen. The results of the histopathological examination of the thyroid follicular cell tumours are consistent with a non-genotoxic aetiology. Overall, it can be concluded that dichlofluanid is a non-genotoxic rat thyroid follicular cell carcinogen.

Rat follicular cell thyroid tumours can arise as a secondary consequence of perturbations in thyroid hormone homeostasis which produce prolonged stimulation of the thyroid gland by thyroid stimulating hormone (TSH) via a positive feedback mechanism [Hill *et al* 1989]. The data obtained from the specialised studies (section 3.3.8) are consistent with a mechanism involving inhibition of thyroid peroxidase by the rat dichlofluanid metabolite thiazolidine-2-thione-4-carboxylic acid. Such inhibition will perturb thyroid hormone homeostasis leading to a prolonged TSH-mediated stimulation of the thyroid gland and eventually to tumour formation. The available *in vivo* data for dichlofluanid indicate that repeated dietary administration for 9 weeks in rats caused a decrease in T3 and T4 levels. Interspecies comparisons of the relative sensitivities of thyroid hormone homeostasis to disturbance by xenobiotics have shown that humans are markedly less sensitive than rats to such disturbances (see section 3.3.8). Overall, it is considered unlikely that the rat thyroid tumours

observed following repeated administration of dichlofluanid are of relevance to human health.

3.3.7 REPRODUCTIVE TOXICITY

3.3.7.1 Multigeneration Studies

Three multigeneration studies are available. Two two-generation studies were OECD and EPA compliant and carried out to GLP. A second two-generation study was carried out as the dose selection in the first was too high. The three-generation study was not carried out to GLP or to any recognised protocol.

In a two-generation study animals (rats Wistar SPF:BOR strain 30/sex/group), received dietary concentrations of 0, 180, 900 or 4500 ppm dichlofluanid (92% purity) throughout the study period. The doses were derived from a two-year chronic toxicity study and an old three-generation reproductive toxicity study.

Animals were treated for 12 weeks and then mated. Dosing continued through mating, gestation and lactation. All litters were reduced to 8 on day 4 post partum and weaned for 3 weeks, macroscopically examined, then sacrificed. A second litter was produced following an identical mating schedule. The F1 adults (30/sex) were taken from the second litter and dosed and mated in the same manner. Offspring from the two matings were treated as above. Necropsies were carried out on the following : animals dying during the study; parental animals; and day 4 litter reduction pups. The achieved compound intakes for parental animals are displayed in Table 3.15.

Table 3.15 : Achieved Compound Intakes (mg kg^{-1} d^{-1}) During The Premating Interval

	F0 parental animals		F1 parental animals	
dose group	**male**	**female**	**male**	**female**
180 ppm	15.7	17.3	19.7	21
900 ppm	86.5	111.7	130.8	145
4500 ppm	591.1	779.4	725	689.2

In the F0 parental generation the following effects were reported. Three females were sacrificed *in extremis*; necropsy revealed that these deaths were not treatment related. At the top dose bloody noses were reported from week 2. From week 5, teeth became white and began to grow rapidly. There were significant decreases in the body weights of males (8 %) and females (11 %) at the top dose, although food consumption was unaffected. The absolute spleen weight was decreased in the top-dose group, by 6 % in males and 14 % in females (statistically significant). The relative liver weight (15 %) and relative testes weights (12 %) were significantly increased in the top-dose group. The relative kidney weights of both sexes

were significantly increased at the top dose (10 % and 7 % males and females respectively). Histopathological examination revealed an increase in the numbers of animals with skull thickening (see Table 3.16). These increases were statistically significant in both sexes at the top dose and the middle dose in males only.

Table 3.16 : Incidence Of Increased Skull Thickness In The Parental Generation (F0)

dose group	males	females
0 ppm	1/30	0/30
180 ppm	4/30	4/28
900 ppm	8/30	3/28
4500 ppm	19/30	24/29

Splenic haemosiderosis was reported in females of the top-dose group only, but was not considered of toxicological significance.

The following effects were reported for pups (F1a/b) of the F0 parental animals. A non significant reduction in numbers was reported in the F1b generation at the top dose only. Pups of the F1a/b generations were reported to show laboured breathing with thin and cold animals found at the top dose (F1a) and middle and top dose (F1b) during lactation. Instances of cyanosis were reported in males (26/240 and 0/240, 4500 ppm and controls respectively) and females (1/240, 7/240 and 9/240 at 0, 900 and 4500 ppm respectively) of the F1a generation. Body weights of F1a pups were significantly lowered throughout lactation at the middle (19.2 % and 17.6 % males and females respectively) and top dose (42 % and 39.7 % males and females respectively) and at the top dose only in the F1b generation (22.6 and 16.8 % males and females respectively).

The index of viability* was significantly lowered in the middle- (4.5 %) and top- (17.2 %) dose groups respectively of the F1a generation, and in the top-dose group of the F1b generation (11.7 %). The index of lactation** was significantly lowered at the top dose of the F1a generation (41 %) and at dose levels >180 ppm in the F1b generation (26 % and 67 % at 900 and 4500 ppm respectively). No pups from litters of the F1b generation were culled at day 4 as there were insufficient numbers to warrant this. The above significant effects were outside the provided reference range. No significant pathology was reported in the F1a or F1b generations.

$$\text{*Index of viability} = \frac{\text{Number of live pups after 4 days (prior to litter reduction)}}{\text{Number of pups born}}$$

$$\text{**Index of lactation} = \frac{\text{Number of live pups after 3 weeks}}{\text{Number of pups after 4 d (after reduction)}}$$

In the F1 parental generation an increase in mortalities was reported in both sexes at the top dose (29/31 and 17/21 males and females respectively), which was considered to be treatment related. Animals dying in the middle group and 3 males and 4 females in the top-dose group were all thin on introduction to the study. At the top dose, bleeding noses were reported at

week 4, continuing until the end of the study, along with increased dental growth after week 10. Food consumption was significantly decreased in the top-dose group from weeks 5-8, after which it was indistinguishable from the control intake. The body weights of females (7.6 %) and the two surviving males (7.2 %) in the top-dose group were significantly decreased.

Necropsy of intercurrent deaths, occurring at 4-5 weeks, in F1 parental animals including the controls revealed red/brown urinary bladder contents, very small black-red areas in the glandular stomach mucosa and red black greasy contents in the jejunum (some instances duodenum or ileum). Histopathological examination revealed erosions of the glandular stomach only. There were no significant effects on the absolute organ weights. The relative kidney weight was significantly increased at dose levels ≥900 ppm (15 and 7 % males and females respectively, at the top dose). A significant increase in the relative spleen weight was reported in males at the top dose (20 %). Histopathological analysis revealed an increased incidence of the finding "focal superficial areas of unossified membrane" in the skull of females, although the shortage of animals at this dose precludes a complete assessment. See Table 3.17. This finding was considered by the study authors to represent a disturbance of bone metabolism.

Table 3.17 : Incidence Of Focal Superficial Areas Of Unossified Membrane

dose group	males	females
0 ppm	4/30	2/30
180 ppm	2/30	0/30
900 ppm	5/30	9/29
4500 ppm	0/2	0/4

Note : an 8.6 % non-significant increase in testes weight was apparent.

The following effects were reported for pups (F2a/b generations) of F1 parental animals. Thin/cold pups were reported in the middle- and top-dose group of the F2b generation and in the top-dose group only of the F2a generation. The birth weights of male F2a pups were significantly reduced at the top dose (6.8 %). The body weights during lactation were decreased (>10 %) at all doses in the F2a generation and at the top dose only in the F2b generation. The small numbers of litters (3 litters only at day 21) in the top dose F2 generation complicates interpretation.

The index of viability was unaffected but the lactation index was significantly lowered at the middle (F2a 18.6 %) and top dose (F2a 50 %) and the top dose only (F2b 25 %). Other abnormalities noted were 2/9 pups in one litter at the top dose, which were found to exhibit vertebral body retardation.

In conclusion, the following NOELs could be set. The NOEL for the F0 parental animals was 180 ppm (equivalent to 15.7 $mg\,kg^{-1}\,d^{-1}$) based on the increase in skull thickness seen at

900 ppm in males. The NOEL for the F1 generation parental animals was 180 ppm (equivalent to 19.7 and 21 mg kg^{-1} d^{-1} for males and females respectively) based on increases in kidney weight and skull effects at 900 ppm. The NOEL for the F1 pups was 180 ppm based on the reduction in body weight at the end of lactation at 90 ppm. The NOEL for F2 pups could not be determined due to the reduced body weight at all doses at the end of lactation. No effects were reported on fertility and therefore, a NOEL of 4500 ppm could be set. No overt developmental toxicity effects were reported. [Unpublished, 1991(b)]

In a further two-generation study, animals (rats Wistar SPF:BOR strain 30/sex/group) received dietary concentrations of 0, 90, or 900 ppm dichlofluanid (90.4-91.1 % purity) throughout the study period. These doses were based on the previous two-generation study.

Animals were treated for 12 weeks and then mated. Dosing continued through mating, gestation and lactation. All litters were reduced to 8 on day 4 post partum and weaned for 3 weeks, macroscopically examined, then sacrificed. A second litter was produced following an identical mating schedule. The F1 adults (30/sex) were taken from the second litter and dosed and mated in the same manner. Offspring from the two matings were treated as above. Necropsies were carried out on the following : animals dying during the study; parental animals; and day 4 litter reduction pups. The achieved compound intakes for parental animals are displayed in Table 3.18.

Table 3.18 : Achieved Compound Intakes (mg kg^{-1} d^{-1}) During The Premating Interval

	F0 parental animals		F1 parental animals	
dose group	**male**	**female**	**male**	**female**
90 ppm	7.3	8.1	9.4	11.4
900 ppm	72	79.4	102.3	117.5

In the F0 parental generation the following effects were reported in animals dying or sacrificed during the study. One male was found dead at 90 ppm and a female receiving 900 ppm was sacrificed after delivering 15 dead pups. Necropsy of these animals revealed no treatment-related effects in the male. In the female necropsy revealed a pale liver with red areas, microscopic black points in the glandular stomach mucosa and dark red mucid contents in the uterine body. Histopathological examination showed suppurative inflammation of the uterus, periacinal coagulative necrosis in the liver, hyperkeratosis and acanthosis of the forestomach, and focal ulcers in the glandular stomach.

In F0 animals surviving until termination, the body weight of males receiving 900 ppm was significantly decreased (4 %) at week 28. No effects were found on macro or histopathology or organ weights at necropsy. Dichlofluanid was also found not to effect insemination, fertilisation or gestation.

The following effects were reported for pups (F1a/b) of the F0 parental animals. With the exception of a decrease in body weight at the end of lactation in the F1 b pups (14.7 %), no adverse effects were found.

In the F1 parental generation the following effects were reported in animals dying or sacrificed during the study. Four males and one females died in the first week post weaning. Necropsy revealed black/red sticky jejunum contents and/ or microscopic black/red points in the glandular stomach in 4 animals. In the fifth animal a gas distended intestine was found. No histopathological data were provided due to autolysis.

On introduction to the study the body weights of F1 parental animals were significantly decreased at 900 ppm for the first week, after which they were indistinguishable from the control animals. No adverse effects were found on macro and histopathological examination. Slight, but significant increases in absolute liver (9 %) and kidney (7 %) weights were reported at 900 ppm. These increases were also noted in relative organ weights. No treatment-related effects on insemination, fertilisation or gestation were found.

The following effects were reported for pups (F2a/b) of the F1 parental animals. With the exception of a decrease in body weight at the end of lactation in the F2 b pups (17 %), no adverse effects were found. No adverse effects were found on macro and histopathological examination.

In conclusion the following NOELs could be set. The NOEL for the F0 parental animals was 900 ppm; equivalent to 72 and 79.4 mg kg^{-1} d^{-1} for males and females respectively. The NOEL for the F1 generation parental animals was 900 ppm; equivalent to 102.3 and 117.5 mg kg^{-1} d^{-1} for males and females respectively. The NOEL for the F1 pups was 90 ppm, based on the reduction in body weight at the end of lactation at 900 ppm. The NOEL for F2 pups was 90 ppm based on the reduction in body weight at the end of lactation at 900 ppm. No effects were reported on fertility and therefore a NOEL of 900 ppm could be set. No overt developmental toxicity effects were reported. [Unpublished, 1992(b)]

A single, poorly reported three-generation study is available in the rat (FB_{30} Elberfeld strain). Animals received dietary concentrations of either 0, 150, 500, 1500, or 4500 ppm dichlofluanid (90.2 % purity) throughout the study period. Parental animals in each generation comprised 10 males and 20 females. Parental animals were mated and allowed to litter down. The first litter from each generation ("a" generation) was culled 4 weeks post weaning, pups from the second litter ("b" generation) containing more than 10 pups were culled on day 5 post partum to 10/litter. The surviving pups were weaned and placed into single sex groups. After 10 weeks parental animals were selected from this group and remaining animals sacrificed; this procedure was repeated for three generations. Pups from the F3b generation were necropsied. The body weights of males from the F0 generation were decreased in the top-dose group until just prior to sacrifice and for the first 4 weeks only in females. In the other two generations of parental animals a significant decrease in body weight was reported at the top dose.

The pups from each generation were found to have no toxicologically significant malformations. There were however effects on birth weight and subsequent weight gain. Pups in the F1b (7.8 %), F2a (6.9 %), F2b (6.4 %) and F3a (6.9 %) generations were significantly lighter both at birth, at the top dose, remaining so at weaning in the top dose group only (F3a pups were also lighter in the lowest treatment group). Pups from the F1a and F3b generations were significantly lighter in the top-dose group only at the end of weaning. Weaned F1b and F2b animals remained lighter than controls. Necropsy of 2 male and 3

female F3b animals did not reveal any toxicologically significant findings. There were no effects on mean pup weight post partum or weaning. Pups in the top-dose group either failed to gain weight or were of low birth weight. No further information was provided, including body weights and active ingredient intake.

No effects were reported on fertility, litter size or lactation performance in any parental generation. The NOELs for parental and fetotoxicity were 1500 ppm, based on reductions in body weight. The NOEL for reproductive toxicity was 4500 ppm. [Unpublished, 1969(a)]

3.3.7.2 Summary Of Reproductive Toxicity

No effects on fertility, gestation or development were found in the three studies.

In the 1992 study no treatment-related effects were found in parental animals. Reductions in pup body weight were reported at 900 ppm from both generations. The NOELs for the F0 parental animals were 900 ppm; equivalent to 72 and 79.4 mg kg^{-1} d^{-1} for males and females respectively. The NOEL for the F1 generation parental animals was 900 ppm; equivalent to 102.3 and 117.5 mg kg^{-1} d^{-1} for males and females respectively. The NOEL for pups was 90 ppm for both generations.

The 1991 two-generation study was carried out at higher dose levels. The major adverse effects were deaths throughout the study period and effects on skull growth. Where NOELs were identified they were lower than those in the 1992 study. In a poorly reported three-generation study NOELs were identified, although higher than those in the 1992 study.

3.3.7.3 Developmental Toxicity

Rat

In the GLP study, rats (Charles River Crl:CD BR ® 28/group) received either 0, 125, 250 or 500 mg kg^{-1} dichlofluanid (91 % purity) by gavage on days 6-15 of gestation. Animals were sacrificed on day 20. The dosing vehicle was 0.5 % v/v aqueous emulophor. No pre-study termination deaths, dose-related pathology or reproductive effects were reported in the dams. No signs of toxicity were recorded. Significant decreases in maternal body weight gain were reported on days 6-8 of gestation in the middle-dose (+3.4 % control compared to treated +0.8 %) and top-dose groups (+3.4 % control compared to treated -1.54 %), which was maintained to study termination in the top-dose group only (+23 % control compared to treated +17.7%).

Food consumption was reduced on day 7 only, but recovered by study termination. No teratogenic or fetotoxic effects were reported, with the exception of a significant decrease in placental weight in the middle-dose group. The maternal NOEL was 250 mg kg^{-1} d^{-1}, based on decreased maternal body weight, and for developmental toxicity was 500 mg kg d^{-1}. [Unpublished, 1989(a)]

A poorly reported study not conducted to GLP is available carried out in the rat (20-23/group strain unknown). Animals received either 0, 30, 100 or 300 mg kg^{-1} dichlofluanid (unspecified purity), in 0.5 % v/v aqueous cremophor, by gavage on days 6-18 of gestation. The study was terminated on day 20 of gestation. Signs of toxicity reported in all treatment groups were diarrhoea, ruffled fur and dyspnoea. Weight gain was decreased in all treated animals but significantly so at the low dose only (12 % and 8.9 % decrease at 30 and 300 mg kg^{-1} respectively). Placental weight was significantly decreased at the top dose (5.3 %); no further treatment-related developmental effects were reported. No further information was provided.

The NOEL for developmental toxicity was 300 mg kg^{-1}. A NOEL for maternal toxicity could not be established due the overt toxicity reported at all doses. [Unpublished, 1974]

Rabbit

A further non-GLP study carried out in the rabbit is available (Himalayan Chbb:Hm 15/group). Animals were administered either 0, 10, 30, or 100 mg kg^{-1} dichlofluanid (89.3 % purity) in 0.5 % v/v aqueous cremophor, by gavage on days 6-18 of gestation. The study was terminated on day 29 of gestation with no deaths being reported during the study. There was a slight decrease in body weight gain (low- and middle-dose groups) on days 6-18 which was statistically significant at the top dose. This was reflected in the terminal body weight of animals in the top-dose group (35 %) when compared to control animals. Food consumption was significantly reduced on days 6-18 in the top-dose group only (compared to controls), increasing to above control levels post dosing in all study groups. Reported reproductive effects were two resorbed litters (one in the middle- and top-dose group). At the top dose numbers of implantation sites and live fetuses were significantly decreased, with two litters being delivered prematurely; no abnormalities were reported.

The NOEL for maternal toxicity, embryotoxicity and fetotoxicity was found to be 30 mg kg^{-1} while the NOEL for developmental toxicity was >100 mg kg^{-1}. [Unpublished, 1982]

3.3.7.3 Summary Of Developmental Toxicity

In three developmental toxicity studies (one rabbit and two rat), one of which was conducted to GLP, dichlofluanid was not found to be a developmental toxicant. In the rat, the NOEL for developmental toxicity was 500 mg kg d^{-1} with a maternal NOEL of 250 mg kg^{-1} d^{-1}, based on decreased body weight. In the rabbit, the NOELs were 30 mg kg^{-1} for maternal toxicity, embryotoxicity and fetotoxicity, and >100 mg kg^{-1} for developmental toxicity.

3.3.8 SPECIALISED STUDIES

The following studies were evaluated in order to provide a mechanistic explanation for the thyroid tumours observed in the rat combined chronic toxicity and carcinogenicity study (section 3.3.5.1) in which an increased incidence of thyroid tumours was observed at the highest dose level of 300-420 mg kg^{-1} d^{-1}.

3.3.8.1 Rat - Thyroid Specific Study

A second 9-week study aimed at elucidating any thyroid specific effects of dichlofluanid is also available. Animals (male SPF Wistar 80/dose) were administered dichlofluanid (92 % purity) at 5 dietary concentrations; either 0, 150, 500, 1500 or 4500 ppm (equivalent to 11.93, 39.3, 120.9, and 355.1 mg kg^{-1} d^{-1} respectively). The ability of the thyroid to concentrate radiolabelled iodine (^{131}I, 37 MBq ml-1) and the levels of circulating thyroid hormones T3 and T4 were determined at 7, 21 and 63 d with 10 animals from each group being used at each time point. A significant decrease in body weight was reported at the top dose at weeks 1, 2, 3 and 9 (4 %), and at 1500 ppm at weeks 2 and 3. Food consumption was also reduced in the top-dose group, by 5 % at week 9. The circulating thyroid hormone levels were significantly reduced at 7 and 21 d. See Table 3.19. At day 63 the levels of both thyroid hormones were not significantly different from control levels. A significant increase in thyroid weight was reported at day 7, after which the thyroid weights of treated animals were indistinguishable from those of control animals. See Table 3.20.

Table 3.19 : Thyroid Hormone Levels During The Study

	T3 (ng 100 ml^{-1})			T4 (mg 100 ml^{-1})		
day	**0**	**4500 ppm**	**%**	**0**	**4500 ppm**	**%**
7	55.2	45.7	-17.3	3.9	2.6	-32
21	52.9	45.3	-14.5	4.2	3.5	-16
63	67.6	61.8	-8.7	5.7	5.1	-11.5

Table 3.20 : Thyroid Weights At Day 7

dose ppm	**control**	**150**	**500**	**1500**	**4500**
weight mg	10	14	13	15	16
% increase	-	40	30	50	60

No effects on iodide accumulation by the thyroid were reported, with the exception of a significant increase at day 7 at 4500 ppm. No deaths or abnormal pathology were reported. However, liver weights were significantly elevated (4 %) in the top-dose group at the termination of the study.

This study has not adequately addressed the question of how dichlofluanid could perturb thyroid hormone homeostasis, leading to tumour formation. [Unpublished, 1981(a)]

3.3.8.2 In Vitro Mechanistic Study

The available toxicokinetic data summarised in section 3.1 identified thiazolidine-2- thione-4-carboxylic acid (TCC), as a major metabolite of dichlofluanid in the rat. TCC belongs to a class of compounds generically known as thionamides which are known to inhibit thyroid peroxidase (TPO) activity. Two well-studied thionamides are propylthiouracil (PTU) and ethylenethiourea (ETU) which have both been reported to modulate thyroid activity in the rat by perturbing thyroid hormone homeostasis via inhibition of TPO functions [Takayama *et al* 1986, Doerge and Tazakawa, 1990. Furthermore, PTU is also reported to affect rat thyroid hormone homeostasis via inhibition of Type 1 5'monodeiodinase activity. TPO and Type 1 5'monodeiodinase enzymes are crucial for the maintenance of circulating triiodothyronine (T3) and thyroxine (T4) levels within the normal physiological range. TPO is located in the thyroid gland and is responsible for sequestering circulating iodide as iodine, mono- and diiodination of the tyrosyl residues on thyroglobulin and the coupling of monoiodotyrosine and diiodotyrosines to produce thyroglobulin bound T3 and T4. These reactions are dependent on the peroxidase activity of TPO to generate the radical intermediates required for the iodination and coupling reactions. Thus, inhibition of TPO will cause a decrease in the plasma levels of T3 and T4. Repeated administration of TPO inhibitors such as ETU will cause an increase in thyroid stimulating hormone (TSH) release from the pituitary gland in an attempt to re-establish thyroid hormone homeostasis. The resulting TSH-mediated hyperstimulation of the thyroid gland, if sufficiently prolonged, eventually leads to thyroid tumours in the rat. Type 1 5'monodeiodinase is located in the liver and other tissues and deiodinates T4 to produce T3, the physiologically active hormone. Inhibition of hepatic Type1 5'monodeiodinase will cause a decrease in the plasma levels of T3. The food colourant erythrosine is known to inhibit this enzyme [Hill *et al* 1989]. Repeated administration of erythrosine to rats has been found to cause thyroid tumours in rats [Borzelleca *et al* 1987], presumably in response to TSH-mediated hyperstimulation of the thyroid gland [Jennings *et al* 1990].

An *in vitro* study was conducted to investigate the potential of TCC to inhibit TPO and Type 1 5'monodeiodinase activity [Unpublished Freyberger 1995]. The following experiments were performed using partially-purified hog TPO preparations in the presence of TCC. TPO-catalysed guaiacol oxidation was determined as a measure of overall peroxidase activity. Other functions of TPO were investigated by measurement of TPO-catalysed iodine formation and TPO-catalysed tyrosine monoiodination. TPO-catalysed metabolism of TCC was also investigated but was of limited scope as no kinetic data were obtained. In all of these experiments PTU and ETU were included for comparison. The potential of TCC to act as a suicide inhibitor was investigated in order to determine whether any irreversible enzyme inhibition occurred. TPO was pre-incubated with excess TCC or a model suicide inhibitor (o-methoxyaniline) and the residual peroxidase activity determined. In addition, the potential of TCC to inhibit hepatic Type 1 5'monodeiodinase activity in a partially-purified preparation from rat liver was also determined. PTU and ETU were again used for comparison.

The results of the study indicated that TCC has the potential to inhibit TPO activity. The IC_{50}s for guaiacol oxidation were ~1000 μM, 45 μM, and >1000 μM for TCC, PTU and ETU respectively. TCC dose-dependently reversibly depressed TPO-catalysed iodine formation, to approximately the same extent as PTU whereas ETU exhibited greater potency than TCC or PTU. These iodine-formation results were presented as absorbance readings and not further

quantified. In relation to TPO-catalysed tyrosine monoiodination, the same order of potency was observed as for TPO-catalysed iodination, i.e. ETU>TCC~PTU. These results were also only presented as absorbance readings. TCC was metabolised by TPO, indicating that TCC was acting as an alternate substrate to iodide or thyroglobulin bound tyrosine. TCC was not found to be a suicide inhibitor of TPO. In the Type 1 5'monodeiodinase inhibition experiment TCC was a weak inhibitor with an IC_{50} of >1000 μM, compared with IC_{50}'s of 0.1 μM and 45 μM for PTU and ETU respectively.

3.3.8.3 Summary of Specialised Studies

Two mechanistic studies are available, the first an *in vitro* study using preparations of TPO and Type 1 5'monodeiodinase enzymes and the second an *in vivo* repeated dose dietary study conducted in the rat. In the *in vitro* study, TCC showed the potential to inhibit TPO-catalysed iodine formation, and TPO-catalysed tyrosine monoiodination. In the 9-week *in vivo* study, significant decreases in plasma T3 and T4 levels were noted on days 7 and 21 of the study at the highest dose level of 355 mg kg^{-1} d^{-1}. On day 63 plasma T3 and T4 levels remained decreased although not achieving statistical significance. A dose-dependent increase in thyroid weight was observed on day 7 of the study only.

It is known that *in vivo*, the initial consequences of inhibition of TPO are a decrease in the plasma levels of T3 and T4 and an increase in TSH release from the pituitary gland in an attempt to re-establish thyroid hormone homeostasis [Hill *et al* 1989]. The resulting TSH-mediated hyperstimulation of the thyroid gland, if sufficiently prolonged, eventually leads to thyroid follicular tumours in the rat. The available data for dichlofluanid indicate that its metabolite TCC may inhibit TPO *in vitro* and that repeated administration causes a decrease in T3 and T4 levels *in vivo*. ETU is an example of a substance which inhibits TPO activity leading to decreased T4 levels and increased TSH levels, and increased thyroid follicular tumours in the rat. Overall, the specialised studies suggest that a possible mechanism by which repeated administration of dichlofluanid to the rat can modulate thyroid activity is perturbation of thyroid hormone homeostasis via inhibition of TPO functions. As with ETU, this mechanism could account for the increase in thyroid follicular tumours in the rat (section 3.3.5.1).

The available evidence suggests that humans are much less responsive to fluctuations in thyroid hormone levels as a result of the presence of a specific plasma T3 and T4 binding protein and a lower level of "normal" activity in the human thyroid gland [Hill *et al* 1989]. In the rat, T3 and T4 are not bound to a specific carrier protein and the "normal" level of activity in the thyroid gland is much greater than that observed in the human thyroid. Furthermore, in situations where the human thyroid is subjected to prolonged hyperstimulation only goitre and not tumour formation have been observed. A review of data relating to prolonged hyperstimulation of the thyroid gland in humans has been published by Hill *et al* (1989). In addition, there is also *in vitro* evidence that TPO activity in primates is refractory to modulation by thionamides [Takayama *et al* 1986] making it less likely that humans would be susceptible to TCC-mediated inhibition of TPO activity.

Overall, the ACP considered that the rat thyroid follicular tumours would not be relevant to human health if they were due to treatment-related hormone imbalance. However it concluded that the *in vitro* studies did not convincingly demonstrate inhibition of thyroid

peroxidase. Whilst it seemed probable that the mechanism was non-genotoxic, a more convincing explanation of the mechanism was required.

3.3.9 HUMAN DATA

Published data [Bjorkner *et-al*, 1990] suggests that occupational exposure to dichlofluanid may cause sensitisation. In a second case study [Hansson C. and Wallengren J., 1995], a woman presenting with contact dermatitis was found upon patch testing to react positively to both dichlofluanid and a dichlofluanid-containing formulation. No further information was provided.

A poorly reported volunteer study is available in which the ability of dichlofluanid (unspecified purity) to cause sensitisation was assessed. Eleven volunteers were dermally exposed to dichlofluanid at 20, 200 and 2000 μg ml^{-1} (as an aqueous suspension). The test solution was applied under occlusive dressings (occasionally non-occlusive) to the back for 24 h. Observations were made 24 and 48 h post application. One positive result was reported (48 and 72 h after application) with 20 μg ml^{-1}. However, this may have been caused by a plaster allergy. It was concluded from this study that dichlofluanid was non-allergenic under the conditions used. No further information was provided. [Unpublished, 1987]

Approval Holders have provided evidence from routine health surveillance suggesting that those employees involved in dichlofluanid manufacture have exhibited no ill health effects. [Unpublished 1989b].

No cases relating to dichlofluanid were reported to HSE under RIDDOR (the Reporting of Injuries, Diseases and Dangerous Occurrences Regulations) in the last 14 years. Similarly, none were identified from EPIDERM (Occupational Skin Surveillance Survey, 1993-1998) or OPRA (Occupational Physicians Reporting Activity, 1994-1998), nor any associated with any antifouling products through the Pesticides Incidents Appraisal Panel. Information provided by the National Poisons Information Service in Scotland identified one enquiry related to an antifouling product containing cuprous oxide and dichlofluanid. No symptoms were reported. A further case related to agricultural use.

3.4 DATA REQUIREMENTS :

i A revised explanation of the mechanism of tumour formation, including a discussion of its relevance to humans.

ii An overview of nephrotoxicity across species, taking into account any data on histopathology and the onset of effects, to determine whether a NOAEL more appropriate to the pattern of user exposure than that established in the 1-year dog study can be identified. If a more appropriate NOAEL cannot be identified from existing data, an oral study of shorter duration in the dog may be required. (see section 4.1.1.)

4. OPERATOR AND 'CONSUMER' EXPOSURE AND RISK ASSESSMENTS

4.1 EFFECT LEVELS IN RISK ASSESSMENTS

A summary of the effect levels for dichlofluanid, identified in the mammalian toxicity assessment, is set out in Table 4.1 :

Table 4.1 : Effect Levels Used In Operator Exposure Calculations

study	route	species	effect levels
acute (dichlofluanid in water)	oral gavage	rat	NOAEL of 1000 mg kg^{-1} based on signs of toxicity at higher dose (apathy, piloerection, increased in water uptake and urine volume, transient drop in body weight).
acute (mineral oil-based formulation)	oral gavage	rat	NOAEL of 24.8 mg dichlofluanid kg^{-1} based on signs of toxicity at higher doses (ataxia, paralysis of hind limbs, gasping, urinary stains).
4-month repeat dose	oral - diet	rat	LOEL was 180 and 341 mg kg^{-1} d^{-1} for males and females respectively based on increase in liver weights in females and in males, a decrease in heart weight and decrease in body weight.
4-month repeat dose	oral - diet	dog	Based on elevated ALT, AST, ALP and blood urea noted in one female (not statistically significant), NOEL estimated as 16.1 mg kg^{-1} d^{-1}.
one-year repeat dose	oral via capsule	dog	Systemic effects on clinical chemistry, haematology and urinalysis parameters at and above 12.5 mg kg^{-1} d^{-1}. NOAEL at 2.5 mg kg^{-1} d^{-1}.
one-year repeat dose	oral	dog	At 1.25 mg kg^{-1} d^{-1} (highest dose tested), both absolute and relative testes weights reduced. However, there was no dose response when compared to higher doses in the above study and weights were within range of historical control values.
two-year repeat dose	oral - diet	dog	At above 34 mg kg^{-1} d^{-1} : changes in clinical chemistry parameters (increased ALP, ALT, bilirubin, cholesterol levels and BSP retention time) indicative of impaired hepatic function; increases in serum creatinine and urea levels and direct measurements of kidney function (decrease in PAH and insulin clearance) suggestive of impaired renal function. Decreases in ovary weight, reported at all doses, precluded the setting of a NOEL.

study	route	species	effect levels
two-year chronic	oral - diet	rat	NOEL estimated to be 218 and 279 mg kg^{-1} d^{-1} (males and females respectively) based on liver and kidney enlargement and slight testicular effects this dose).
two-year chronic	oral - diet	mouse	NOAEL 252 and 273 mg kg^{-1} d^{-1} (males and females respectively) for non-neoplastic events.
two-year chronic/ carcinogenicity	oral - diet	rat	NOEL 54.4 and 73.1 mg kg^{-1} d^{-1} (males and females respectively) for neoplasms (increase in thyroid tumours). Effects of excess fluoride and cranial osteosclerosis noted at lowest dose (9.4 - 13.5 mg kg^{-1} d^{-1})
two-year carcinogenicity	oral - diet	mouse	LOAEL 50.1 and 63.7 mg kg-1 d-1 (males and females respectively) based on increases in teeth/bone fluoride levels and leukocyte numbers.
2-generation	oral - diet	rat	NOEL (F_0 generation) 15.7 mg kg^{-1} d^{-1} based on increased skull thickness in males
2-generation	oral - diet	rat	No treatment-related effects were observed at dose levels of up to 72 mg kg^{-1} d^{-1} (the NOEL).
3-generation	oral - diet	rat	NOEL (F_0 generation) 1500 ppm based on reductions in body weight.
developmental toxicity	oral - gavage	rat	maternal NOEL 250 mg kg^{-1} d^{-1} based on decreased body weight. NOEL for developmental toxicity was 500 mg kg^{-1} d^{-1}.
developmental toxicity	oral - gavage	rat	Maternal NOEL could not be established due to overt toxicity at all doses. NOEL for developmental toxicity was 300 mg kg^{-1}
developmental toxicity	oral - gavage	rabbit	Maternal NOEL 30 mg kg-1. NOEL for developmental toxicity was >100 mg kg^{-1}.

4.1.1 DISCUSSION OF EFFECT LEVELS USED IN THIS RISK ASSESSMENT

In the chronic studies, the most consistent findings were cranial osteosclerosis in the rat, and findings consistent with fluorosis in a 2-year mouse study. Clinical chemistry findings with supportive histopathology were observed in a 1-year study in the dog which were indicative of renal and liver damage.

A detailed assessment of cranial osteosclerosis was performed in a 2-year study in the rat; a LOEL was established at 10-14 mg kg^{-1} d^{-1}. There was a clear increase in the incidence of cranial osteosclerosis in the low- and middle-dose groups, with almost all animals affected at the top dose. These findings are likely to be secondary to fluorosis in these animals. In terms of interspecies comparisons, in a mouse 2-year study, thickening of both the appositional bone of the cranial vault and nasal turbinates, and tooth alveolitis were observed at the top dose of 1,731-1,873 mg kg^{-1} d^{-1}. These findings are also considered to be a secondary

consequence of fluorosis in these animals. No evidence of fluorosis was observed in the dog studies. . In this study a NOAEL of 2.5 mg kg^{-1} d^{-1} was established with nephropathy reported at 12.5 mg kg^{-1} d^{-1}.

Dichlofluanid was found to cause an increase in the incidence of thyroid tumours at highest dose level of 300-420 mg kg^{-1} d^{-1}. These tumours occurred at a single site, were of late onset and generally benign pathology. These pathology findings are indicative of a non-genotoxic aetiology, consistent with the overall conclusions from the genotoxicity studies. Rat thyroid tumours arise as a secondary consequence of perturbations in thyroid hormone homeostasis, leading to prolonged stimulation of the thyroid gland via a positive feedback mechanism. Interspecies comparisons of the relative sensitivities of thyroid hormone homeostasis to disturbance by xenobiotics have shown that humans are markedly less sensitive than rats. Overall, the ACP considered that the rat thyroid follicular tumours would not be relevant to human health if they were due to treatment-related hormone imbalance. However it concluded that the *in vitro* studies did not convincingly demonstrate inhibition of thyroid peroxidase. Whilst it seemed probable that the mechanism was non-genotoxic, a more convincing explanation of the mechanism was required.

It is considered that cranial osteosclerosis is likely to represent a fluoride mediated perturbation of bone metabolism but is not considered to be of concern for human health. It is also considered that the observed renal and liver damage is of concern to human health. For professional operators the ACP considered that the most appropriate NOAEL to use in risk assessments was the figure of 2.5 mg kg^{-1} d^{-1} taken from the one year dog study. Professional operators would not be expected to use products for prolonged periods, therefore a value from a one year study would be suitable. The other reported end points (thyroid tumours and osteosclerosis were associated with chronic exposure and much higher doses. Acceptable risk assessments would require a safety margin of about 100 over the NOAEL 2.5 mg kg^{-1} d^{-1} . Consequently these would also offer a high margin of safety against the longer term end points.

Although amateur users are expected to use products very infrequently sub acute, rather than acute studies, will be used for identification of NOAELs, since these studies address a wide range of toxicological end-points and include histopathological assessment. No suitable sub-acute studies have been provided for dichlofluanid and therefore the NOAEL of 2.5 mg kg^{-1} d^{-1} from the one year dog study will be used as the preferred alternative.

Dichlofluanid is of moderate to low acute inhalation toxicity (LC_{50} 1233-1238 mg m^{-3}).No repeat dose inhalation studies are available, therefore the risk assessment for inhalation toxicity will use this LC_{50} value.

No information is available on the penetration of dichlofluanid through skin; for the purposes of this risk assessment a value of 10 % for dermal penetration has been assumed.

Dichlofluanid is not classified as a skin or eye irritant.

The substance is a potential skin sensitiser.

In its wider consideration of the booster biocide reviews the ACP concluded that when considering the risks for amateur users using antifouling products classified as skin

sensitisers it was important to take account of the quality of the data, in particular the evidence of a risk to people and the likely pattern of use. The ACP also noted that the products can contain rosin, a co-formulant present in most antifouling products. Rosin is classified as a skin sensitiser and is widely used in other products available to the general public. It concluded that amateur application by roller brush or spreader could continue where there were no, or only very rare, reports of skin sensitisation or severe irritation in humans and where the use of the active ingredient or coformulant (eg rosin) had been fairly widespread. The ACP considered that there would be a greater concern associated with the application by spray or aerosol of products containing a booser biocide classified as a skin sensitiser and that generally these should not be permitted.

In applying these criteria to dichlofluanid, the ACP noted that animal data showed that dichlofluanid should be classified as a skin sensitiser but there were very few reports of skin sensitisation in humans. In addition the ACP noted that there were no reports of skin sensitisation in workers involved in the manufacture of dichlofluanid. Consequently it considered that amateur application by roller brush or spreader could continue with the recommendation that users where gloves as a precautionary measure. Risks were not acceptable for amateur spray application.

4.2 OPERATOR AND CONSUMER EXPOSURE

4.2.1 INFORMATION RELATING TO USER EXPOSURE

Risk assessment patterns of use data are based on surveys and anecdote. They are as accurate as is possible, but should not be regarded as definitive.

4.2.1.1 Industry Data

An industry consortium has submitted data for consideration : an operator exposure study, 'Determination of exposure to tin during commercial application of antifouling paint to ship hulls' [Unpublished, 1995].

4.2.1.2 HSE Data

Since 1994 the Health and Safety Executive (HSE) has gathered information on human exposure to antifouling products in the professional and amateur sectors, to inform its role of assessing exposure and risk to operators and others. The information takes two forms :

- the pattern of work :-the frequency and duration of potential exposure, the areas coated per session, the amount of product used and seasonal factors

- the exposure :-the median and realistic worst case exposures in applying the products and identified tasks or jobs.

The surveys and studies informing HSE assessments are :

- 9 surveys applying copper-based antifoulant to ships [40 exposure data; HSE, 1994]
- 5 surveys applying tin-based antifoulant to ships [20 exposure data; HSE, 1996]
- 4 surveys applying various antifoulant to ships [10 exposure data; IOM 1996]
- pattern of use survey [2 commercial organisations, 4 service organisations for leisure craft; HSE 1994/5]
- 8 surveys applying copper-based antifoulant to leisure craft [9 exposure data; HSE, 1997/9]

All HSE surveys took place in the north of England or Scotland. Some information on patterns of use was derived through exposure surveys. Each exposure data point comprised the potential dermal exposure (the amount of antifoulant product depositing on the outer surface of the person), the exposure of hands inside protective or other gloves, exposure by inhalation, the tasks done and the amount of product used.

HSE holds no information on the relative market importance of products or active substances. Three-quarters of the products found being used in the 1994/5 survey were 'conventional or contact leaching' (the active ingredient leaches from the antifoulant) and one-quarter 'ablative' (the active ingredient is bound in a copolymer antifoulant which hydrolyses slowly in sea water). HSE has no reliable data relating to exposures in the military sector, where data relate only to airborne concentrations of copper. There are no data for other immersed structures (e.g. oil-rigs, jetties, fish-farm installations), nor on exposure in stripping expired antifoulings.

4.2.1.3 Environment Agency Data

The Environment Agency [1998] commissioned a report on environmental problems from antifouling agents that contained information on patterns of use.

4.2.1.4 Expression Of Exposure Data

All HSE data are quoted in terms of the antifoulant product being applied and are time-weighted. Data are therefore normalised and in the forms 'mg product h^{-1}' for dermal exposure and 'mg product m^{-3}' for inhalation exposure, respectively. The sampling methods for potential dermal exposure using patches have been validated for spraying activities [Unpublished, 1996. Unpublished, 1998]. However, they have not been validated for painting or paint handling.

It is inappropriate to express 'exposure' simply as a single value. In fact, there are exposure distributions. However, data are sparse due to the difficulty and high cost of their acquisition, and the exact nature of the distribution cannot be proven. Consequently, complex statistical treatments are considered inappropriate. HSE statisticians gave their opinion that in cases where data are sparse, it is valid to consider in detail only the non-zero results. Results with an effective value of zero can be taken into account in the 'frequency' or chance that exposure will occur.

The median value in a distribution, moderated by this frequency, represents a 'central tendency' value. A realistic worst case is represented by the 95th percentile data point of non-zero values, or if the distribution data are sparse, by the highest values found. Where the highest data point is a clear outlier (e.g. many times higher than the next highest point), a decision can be taken to note that datum but disregard it for the purposes of risk assessment.

4.2.1.5 Mitigation Of Exposure To Antifoulants

Work clothing, whether or nor constituting formal personal protective equipment, does have protective properties. Impermeable clothing (e.g. Tyvek suit) will stop liquids reaching the skin, but easily becomes contaminated inside and is difficult to clean properly. Furthermore, deposits may concentrate on the equipment surface and are available for dislodging. Cloth-based equipment will tend to retain liquids and dusts to some extent; there will also be run-off. Penetration of clothing occurs through liquids soaking through seams, zips and elasticated parts, through being rubbed through by frequent contact and by being drawn through openings at neck, wrist and ankle by the 'bellows effect' when the operator moves inside the clothing. HSE data indicate a median 4 % penetration of the outer layer of work clothing, generally a coverall. Having more than one layer of work clothing will provide better protection.

Protective gloves are generally effective in protecting the hands from antifoulant. However, hand contamination is inevitable, and the exposure route may be through putting on and taking off used gloves. One measure to mitigate continuing exposure through protective gloves being contaminated inside is for regular replacement of protective gloves, for example following each antifouling job.

Respiratory protective equipment (RPE) needs to achieve two objectives : to mitigate exposure by inhalation and to protect the skin of the face, head and neck. In general, a high standard of respiratory protective equipment is needed for sprayers and possibly for some ancillary workers, with a workplace protection factor of at least 50. The person at greatest risk is the sprayer, and for spraying, such protective equipment should be mandatory. If the pot-men were to work in the vicinity of the spray plume, then they should wear appropriate protective equipment. Appropriate equipment would be of a standard equivalent to an FFP3 disposable filtering facepiece respirator or better. However, the need for respiratory protection for workers other than sprayers is considered to be a matter for the COSHH workplace risk assessment. It is considered unlikely that amateur users would be able adequately to select and use RPE.

4.2.1.6 Factors Affecting Exposure Estimates For The Use Of Antifouling Products

When the partial reviews of the physical chemistry and mammalian toxicity of other booster biocides were presented to the ACP in 1999, Members expressed concern over the apparently low values of the toxicity:exposure ratios (TERs) derived for a number of compounds. They agreed that the assessment of risk to users and bystanders should be revised to take into account all factors affecting exposure and increase the clarity and transparency of the risk assessments. The TERs of particular concern related to the high end exposures (95^{th} percentile or higher) that were calculated from the operator exposure model. For antifouling applications, the high end predictor is based on the 95^{th} percentile for sprayers and the worst case results for pot-men.

Dermal Exposure

HSE believes the antifouling model to be an accurate predictor of the amount of product that deposits on the outer surfaces of a worker's clothing; as a worst case this amounts to in excess of 120 g of product in a spray session. The amount available for uptake following transfer to the skin is another matter. The estimation of systemic dose, resulting from conditions that can lead to 120 g of paint on the outside of clothing is open to interpretation and requires a degree of professional judgment to reach a realistic conclusion. In addition, the dermal absorption value used in the risk assessment is also critical to the resulting estimate of systemic dose.

Extent Of Penetration Through Clothing

HSE data indicate that 4 % is a realistic figure to adopt for the penetration of antifouling products through a single layer of typical protective workwear. Information from a number of sources suggests that the performance of protective clothing may be related to the level of the challenge. Laboratory experiments indicate that the higher the challenge, the lower the proportion of product that will penetrate to become available as a potential source for contact with the skin. This phenomenon has also been observed during field studies, such as those reported by the Institute of Occupational Medicine [1994] during the investigation of sheep dipping practices and field-effectiveness of PPE. Further layers of clothing will provide an extra barrier to skin contact.

Custom within the antifouling industry, brought about by a recognition that formulations may be unpleasant to work with and are difficult to remove from the skin, is for operators to protect themselves well, and often to wear two sets of coveralls. HSE is prepared to accept that it may be possible, at the higher levels of contamination, to consider a penetration factor through coveralls and clothing, and then onto the skin, at about 1 %. The value for penetration at 1 % is seen as the practical lower limit for modeling purposes as there will always be the potential for contact of product with exposed skin (e.g. around wrists, face and neck and through handling previously contaminated clothing). The ability of protective clothing to reduce potential exposures by at least two orders of magnitude has been demonstrated in the studies by the IOM [Unpublished 1996} and is supported by the experimental findings related to penetration compared to challenge which are built into the POEM model. [MAFF, 1992].

Extrapolation From Clothing To Skin To Systemic Dose

The modeling process is not very good at estimating how much of the product finds its way to the skin, and how much of the active substance in the product is eventually absorbed. The current HSE estimates are precautionary and based on all of the product that is predicted to penetrate the layers of clothing getting to the skin, and a quantity of the active substance within the product immediately penetrating through to become a systemic dose. The amount of uptake via the skin may be established through dermal absorption studies, or if not available by using a default value such as 10 %. However, such uptake is considered unlikely to happen in reality for a number of reasons :

- only a proportion of what lands on the skin is available to be absorbed when product deposition occurs in the form of spots or blobs as active substance will be contained within the matrix of the dried-on product;
- the model does not take into account the dynamics of deposition or absorption, nor the kinetics of metabolism;
- the model does not take into account actions to remove residues after work, particularly from the hands.

Patterns Of Exposure

A further factor relates to interpretation of the findings on exposure. Account needs to be taken of the pattern of work for a painter and the pattern of use of any particular product. Professionals spend much of their time carrying out preparatory work during vessel refitting - many other jobs take place while a vessel is in dry dock and it may be there for a number of weeks. Consequently, exposure to antifouling paints is irregular with long intervals between exposure. Exposure to one particular active substance, other than copper compounds, is even less frequent. The most realistic worst case exposure scenario is that a painter may be exposed for no more than two or three days a month, but not every month, and then not to the same active substance. High end exposures do not occur every time and could be considered as acute.

Hand Exposure

Estimates of hand contamination play an important part in development of the exposure assessment for antifoulants. This is particularly true for the pot-men who come into contact with large amounts of product while of replenishing reservoirs for sprayers. There is always the opportunity for spillage. Where pot-men have worn suitable and adequate new gloves, it appears the levels of contamination to the hands have generally been low. Where pot-men have worn inappropriate gloves, old gloves, or no gloves at all, elevated exposures have been registered. These elevated exposures heavily influence the model. Appropriate changing of gloves suggests that hand exposure can be reduced.

For the sprayer, hand exposures tend to be lower than for the pot-man and are not a main driver of the exposure estimate.

Inhalation Exposure

Sprayers will always need to wear respiratory protective equipment. For the pot-men, exposure to spray aerosol is intermittent and unusual. Results indicate that the normal range (18 of 19 results) is between 0.2 and 4.0 mg m^{-3} of product. One exceptional result (the highest recorded) has been recalculated at 24 mg m^{-3} (previously 42 mg m^{-3}, but closer inspection of the proportion of active material in the paint has caused abatement of this particular result). However, it is considered to be unlikely that even a result of this magnitude would be a true reflection of the personal exposure of a pot-man to aerosol. The individual result showed no elevated potential dermal exposure. HSE has judged that, when considered in the context of the other samples within the data set, the specific result of 24 mg m^{-3} did not reflect exposure to inhalable aerosol; the result was more likely to have arisen through direct transfer of antifoulant, and possibly by direct contamination.

4.2.2 EXPOSURE ASSESSMENT - ANTIFOULANT SPRAYING

Industry data are taken from the study 'Determination Of Exposure To Tin During Commercial Application Of Antifouling Paint To Ship Hulls'.

The following data are taken from HSE (2000) taking account of the Environment Agency technical report (Environment Agency, 1998). For roller and brush applications, data presented Garrod *et al* (2000) have been assumed to apply to professionals.

4.2.2.1 Patterns Of Use In Spray Operations - Professional Users

Industry information on patterns of use accords reasonably well with existing knowledge and reflects current practice.

The work involves dry-docking for vessels the size of tugs and above, or docking on a hard surface for small fishing vessels. During the time for removing and re-applying antifouling, general overhaul and refitting takes place. Consequently, applying the antifouling is a minor

proportion of the time spent working on the vessel. Workers remove and apply surface coatings over the ship, (e.g. bilges, holds), using for example, two-pack epoxy preparations. The pattern-of-use survey indicated that up to 10 % of employees' time might be spent in working with antifoulings.

Professionals work year-round. The vessel is cleaned with a high-pressure water-jet (for self-polishing coatings) or with abrasive grit (for erodable coatings). Bare metal surfaces are prepared with coatings such as corrosion inhibitors. The antifouling is then applied using airless spray techniques at up to 100 bar. Sufficient sprayers are employed to ensure that one coat is applied in one work day. Rarely are more than two coats applied.

Applying antifoulant requires 2 to 4 persons per spray position. The three identified tasks are :

- spraying :- the sprayer,
- mixing and loading :- the pot-man (who prepares the antifoulant and ensures its supply to the high pressure pump),
- ancillary :- the rein or tender men, who attend to keeping paint lines free and may also manoeuvre the mobile access platform (cherry-picker).

Antifoulings are applied on several days a month, for no more than two consecutive days a week. There would normally be one coating session per day. In the HSE surveys the duration of daily work ranged from 40 to 360 minutes per coating session (median 184 minutes), for each of the three identified tasks.

There are only estimated data for the quantity of antifoulant used per spray session. The quantity used ranged from 25 to over 800 litres of antifoulant (median 240 litres). The vessel surface areas coated ranged between 600 and 4000 m^2 (median 1600 m^2).

Where safety data sheets are supplied with products to professional end-users, this supply is often through the ship owner, who does not necessarily transmit the data sheet. Contractors rely on a compendium of data sheets which may become outdated. Contractors were found to have a default set of equipment, risk assessments and personal protective equipment that they used for most situations. In all surveys, overalls and gloves were found to be available for use, although these items were not always ‘suitable’. Respiratory protective equipment was always found to be available. Sprayers usually took steps to protect any exposed skin from antifoulants.

4.2.2.2 Exposure Data For Spray Operations - Professional Users

Detailed calculations are presented in Appendix1. These are based on a 3-hour shift and (as outlined in paragraph 4.2.1.6 - extent of penetration through clothing), a value of 4 % for penetration of a single layer of work clothing and a value of 1 % for penetration of a double layer of work clothing, for sprayers, pot-men and other professional operators such as tenders. Exposure data are quoted as in-use concentration of antifouling product on skin and inhaled.

Industry data were reported in terms of the tin content of the product applied. The amounts of tin measured were extrapolated back to an estimate of product exposure. The Industry and HSE estimates of potential dermal exposure may be compared (mg product/hour) :

	Industry		**HSE**	
	central tendency	maximum	central tendency	maximum
sprayer	10500	49800	6170	44700
pot-man	1950	6550	2940	15000

The data are sufficiently similar to give confidence that the Industry study and HSE survey data overlap, despite the different sampling media used to measure potential dermal exposure. This suggests that the Industry study and the HSE surveys belong to the same distribution. Consequently, no separate risk assessment will be conducted on the Industry data.

4.2.2.3 Patterns Of Use In Spray Operations - Amateur Users

There is no information that amateurs use spraying to apply antifouling, though this mode of application may be permitted in the conditions of approval. Clearly, spraying of leisure-craft would be of shorter duration than the median around 3 hours, which is typical for professionals. In contrast with application by brush and roller, (section 4.2.5.2.2), there is often a clear need for respiratory and skin protection in spray application of antifoulants and it is unlikely that amateurs would be able to select or use personal protective equipment adequately. This suggests that approval of application of antifoulants by spraying by amateurs may be inappropriate.

4.2.2.4 Exposure Data For Spray Operations - Amateur Users

While there are no directly related exposure data, the data model relevant to professional sprayers should apply, assuming no RPE. Amateur sprayers are assumed to wear a coverall and therefore, clothing penetration of 4 % has been used in calculations (unlike amateur use of brush and roller in section 4.2.4.2).

4.2.3 SYSTEMIC EXPOSURES IN SPRAY OPERATIONS

4.2.3.1 Calculations

The data sets and exposure calculations are set out in Appendix 1.

Assumptions :

- 10 % active substance in the product,
  10 % dermal penetration default,
  4 % or 1 % clothing penetration

Exposure estimates for all workers are summarised in Tables 4.2 to 4.8. Calculations are presented for clothing penetration of 4 and 1 %, for standard workwear and additional PPE respectively as explained in section 4.2.1.6. The calculation for professional sprayers includes respiratory protection by air-fed RPE with a 50-fold protection factor. For amateur

spraying, calculations are presented in Table 4.8, with 4 % clothing penetration as explained in section 4.2.2.4.

Table 4.2 : Contact And Systemic Exposure To Dichlofluanid For Professional Sprayers - 4 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[3 hour job, 4 % clothing penetration, 6170 mg h^{-1} central tendency, 44700 mg h^{-1} 95th percentile, 60 (median) & 241 (worst case) mg h^{-1} of product in-glove]*	920	6080
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	92	608
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**9**	**61**
Intake of product by inhalation **with RPE** (mg d^{-1}) *[3 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 6 mg $^{m-}3$, worst case 64.6 mg m^{-3} of product]*	0.45	4.84
In air concentration of dichlofluanid without RPE mg m^{-3}	0.6	6.46
In air concentration of dichlofluanid with RPE mg m^{-3}	0.012	0.13
Amount of dichlofluanid inhaled (mg d^{-1})	**0.05**	**0.5**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1}) wearing RPE	**0.15**	**1.03**

Table 4.3 : Contact And Systemic Exposure To Dichlofluanid For Professional Pot-Men - 4 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[3 hour job, 4 % clothing penetration, 2940 mg h^{-1} central tendency, 15000 mg h^{-1} 95th percentile, 35 (median) & 1380 (worst case) mg h^{-1} of product in-glove]*	458	5940
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	45.8	594
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**4.6**	**59.4**
Intake of product by inhalation (mg d^{-1}) *****, without RPE. *[3 hour job, 1.25 m^3 h^{-1} inhaled volume,, central tendency 0.6 mg m^{-3}, realistic worst case 3.84 mg m^{-3} of product]*	2.25	14.4
In air concentration of dichlofluanid without RPE mg m^{-3}	0.06	0.38
In air concentration of dichlofluanid with RPE mg m^{-3}	N/A	N/A
Amount of dichlofluanid (mg d^{-1})	**0.23**	**1.4**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	**0.08**	**1.01**

***** excludes top data point which is considered an outlier. This operator had significantly lower dermal exposure (by a factor of between 10 and 30) than each of the others in the study and his inhalation exposure was higher than that recorded for his associated sprayer-this would not be expected and cannot be explained. HSE concluded the sample has become contaminated and, consequently, the point discarded.

Table 4.4 : Contact And Systemic Exposure To Dichlofluanid For Other Professional Operators (Ancillary Workers) - 4 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[3 hour job, 4 % clothing penetration, 885 mg h^{-1} central tendency, 3470 mg h^{-1} 95th percentile, 35 (median) & 180 (worst case) mg h^{-1} of product in-glove*	211	956
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	21	95.6
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**2.1**	**9.6**
Intake of product by inhalation (mg d^{-1}) *[3 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.8 mg m^{-3}, realistic worst case 4.8 mg m^{-3} of product]*	3	18
In air concentration of dichlofluanid without RPE mg m^{-3}	0.08	0.48
In air concentration of dichlofluanid with RPE mg m^{-3}	N/A	N/A
Amount of dichlofluanid inhaled (mg d^{-1})	**0.3**	**1.8**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	**0.04**	**0.19**

Table 4.5 : Contact And Systemic Exposure To Dichlofluanid For Professional Sprayers - 1 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[3 hour job, 1 % clothing penetration, 6170 mg h^{-1} central tendency, 44700 mg h^{-1} 95th percentile, 60 (median) & 241 (worst case) mg h^{-1} of product in-glove]*	365	2060
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	36.5	206
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**3.7**	**21**
Intake of product by inhalation **with RPE** (mg d^{-1}) *[3 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 6 mg m^{-3}, worst case 64.6 mg m^{-3}, of product]*	0.45	4.84
In air concentration of dichlofluanid without RPE mg m^{-3}	0.6	6.46
In air concentration of dichlofluanid with RPE mg m^{-3}	0.012	0.13
Amount of dichlofluanid inhaled (mg d^{-1})	**0.05**	**0.5**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1}) wearing RPE	**0.06**	**0.36**

Table 4.6 : Contact And Systemic Exposure To Dichlofluanid For Professional Pot-Men - 1 % clothing penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[3 hour job, 1 % clothing penetration, 2940 mg h^{-1} central tendency, 15000 mg h^{-1} 95th percentile, 35 (median) & 1380 (worst case) mg h^{-1} of product in-glove]*	193	4590
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	19	459
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**1.9**	45.9
Intake of product by inhalation (mg d^{-1}) *****, without RPE. *[3 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.6 mg m^{-3}, realistic worst case 3.84 mg m^{-3} of product]*	2.25	**14.4**
In air concentration of dichlofluanid without RPE mg m^{-3}	0.06	0.38
In air concentration of dichlofluanid with RPE mg m^{-3}	N/A	N/A
Amount of dichlofluanid inhaled (mg d^{-1})	**0.23**	**1.44**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	**0.04**	**0.79**

***** excludes top data point which is considered to be an outlier. This operator had significantly lower dermal exposure (by a factor of between 10 and 30) than each of the others in the study and his inhalation exposure was higher than that recorded for his associated sprayer-this would not be expected and cannot be explained. HSE concluded the sample has become contaminated and, consequently, the point discarded.

Table 4.7 : Contact And Systemic Exposure To Dichlofluanid For Other Professional Operators (Ancillary Workers) - 1 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[3 hour job, 1 % clothing penetration, 885 mg h^{-1} central tendency, 3470 mg h^{-1} 95th percentile, 35 (median) & 180 (worst case) mg h^{-1} of product in-glove]*	131	644
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	13	64.4
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**1.3**	**6.5**
Intake of product by inhalation (mg d^{-1}) *[3 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.8 mg m^{-3} realistic worst case 4.8 mg m^{-3} of product)*	3	18
In air concentration of dichlofluanid without RPE mg m^{-3}	0.08	0.48
In air concentration of dichlofluanid with RPE mg m^{-3}	N/A	N/A
Amount of dichlofluanid inhaled (mg d^{-1})	**0.3**	**1.8**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	**0.03**	**0.14**

Table 4.8 : Contact And Systemic Exposure To Dichlofluanid For Amateur Sprayers - 4 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[1 hour job, 4 % clothing penetration, 6170 mg h^{-1} central tendency, 44700 mg h^{-1} 95th percentile, 60 (median) & 241 (worst case) mg h^{-1} of product in-glove]*	306	2030
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	30.6	203
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**3**	**20**
Intake of product by inhalation **without RPE** (mg d^{-1}) *[1 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 6 mg m^{-3}, worst case 64.6 mg m^{-3} of product]*	7.5	80.7
In air concentration of dichlofluanid without RPE mg m^{-3}	0.6	6.46
Amount of dichlofluanid inhaled (mg d^{-1})	**0.75**	**8**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	**0.06**	**0.5**

4.2.3.2 Assessment Of Risks During Spraying

The levels of exposure estimated for professional and amateur workers have been used to calculate the toxicity:exposure ratios (TERs) set out in Table 4.9.

Table 4.9 : TERs For Professional And Amateur Users During Spraying

user	central tendency exposure (mg kg^{-1} d^{-1})	TER	worst case exposure (mg kg^{-1} d^{-1})	TER
professional users (NOAEL 2.5 mg kg^{-1} d^{-1})				
4 % clothing penetration				
sprayer (with RPE)	0.15	17	1.03	2
pot-man	0.08	31	1.01	2
ancillary worker	.04	63	0.19	13
1 % clothing penetration				
sprayer (with RPE)	0.06	42	0.36	7
pot-man	0.04	63	0.79	3
ancillary worker	0.03	83	0.14	18
amateur users (NOAEL 2.5 mg kg^{-1} d^{-1})				
	0.06	42	0.5	5
inhalation exposure (LC_{50} 1223-1338 mg m^{-3})				
sprayer(with RPE)	0.012 mg m^{-3}	~100,000	0.13 mg m^{-3}	~9000
pot-man	0.06 mg m^{-3}	~20,000	0.38 mg m^{-3}	~3000
ancillary worker	0.08 mg m^{-3}	~15,000	0.48 mg m^{-3}	~2500
amateur	0.6 mg m^{-3}	~2000	6.46 mg m^{-3}	190

4.2.3.1 Professional Users

When comparing exposure with the NOAEL 2.5 mg kg^{-1} d^{-1}, the TER values for professional operators wearing PPE and RPE are < 100 and give some cause for concern. However the values are derived using a default skin penetration value of 10%. In addition, users may only be exposed to dichlofluanid infrequently, given the typical patterns of use for these products. The ACP therefore considered that the risk assessment was conservative and that uses could continue provided that data on the dermal penetration of dichlofluanid from formulations representative of approved antifouling products were generated in order to refine the risk assessment. The risk assessments for inhalation exposure (comparing exposure with the LC_{50} of 1223-1338 mg m^{-3})were acceptable.

In addition to the PPE specified previously, it is also proposed that professional users should be required to wear impervious footwear which protects the lower leg. This is good hygiene practice and is consistent with the requirements for other active ingredients used in antifouling products.

Hooded air-fed respiratory protective equipment with a nominal protection factor of 50 or more is needed for the sprayer and will also protect potentially exposed face and neck skin from contact. Other workers' need for RPE should be determined by the employer through the workplace COSHH assessment.

With regard to skin effects, it is assumed that if contact occurs then operators will be at risk. Workers should have a default set of protective equipment which would include coveralls of a contrasting colour to the product being applied (worn beneath a disposable hooded coverall), protective gloves, and impervious footwear that protects the lower leg. To mitigate top-end exposure to hands, the protective gloves should be discarded at the end of each antifouling spray job (i.e. the gloves worn for one or two days only).

4.2.3.2 Amateur Users

Though amateur products have been approved for spray application, there is no information to indicate if this occurs. The calculated TERs indicate some concern for systemic toxicity and for inhalation exposure. In addition, the ACP, during its consideration of skin sensitisation of substances used in antifouling products (see section 4.1.1), concluded that amateur application by roller brush or spreader could continue where there were no, or only very rare, reports of skin sensitisation or severe irritation in humans and where the use of the active ingredient or coformulant (eg rosin) had been fairly widespread. However it considered that there would be a greater concern associated with the application by spray or aerosol of products containing a booser biocide classified as a skin sensitiser and that generally these should not be permitted.

4.2.3.3 Assessment Of Risks During Application By Aerosol

Approvals have been granted for antifouling products containing up to 1.5 % w/w dichlofluanid for application by ready-for-use aerosol by amateurs and professionals. HSE has no data on application of antifouling products by aerosol but considers that aerosol spraying would be of short duration and not repeated. However, there are no reliable exposure data available. Extensive spraying by amateurs using an aerosol (maximum pack size 250 ml) is not anticipated but could occur.

As noted in parag 4.2.3.2, the ACP considered that approval for amateur application of aerosol products should be revoked.

4.2.4 EXPOSURE ASSESSMENT - ANTIFOULANT APPLICATION BY BRUSH, ROLLER OR SPREADER.

The following data are taken from HSE (2000) and Garrod *et al* (2000). Approvals have been granted for antifouling products containing dichlofluanid for application by spreader by amateurs and professionals. HSE has no data on application of antifouling products by spreader. However it is possible that painting and spreading take place consecutively. Exposure in spreading is likely to be similar to that in painting - principally to the hands, with legs receiving the greatest proportion of exposure thereafter, and exposure by inhalation, minimal. There is no informaton on the typical duration of a spreading job, but it is unlikely to exceed that for painting by a large margin.

4.2.4.1 Patterns Of Use For Brush And Roller application - Amateur And Professional (Chandlers) Users

The HSE's information is that the work is seasonal (springtime), and may be performed on a slip-way, hard-standing or chandler's yard. Boat owners comprise the great majority of users, with a very small proportion of boats being treated with antifoulant by chandlers. The boat is cleaned with a high-pressure water-jet and may be scraped. The antifouling is applied using paint brush or paint roller. Rarely are more than two coats applied. Applying antifoulant employs no more than 2 persons per vessel. While amateur products have been approved for spraying, there is no information to indicate that this happens. Nor is there any information on the use of aerosol spray packaged antifoulant products for spot usage. The quantity of antifouling used per application session was found to range between 1.5 and 5 litres (median 4 litres; these were all copper-based products). Normally, 2 coats were applied to boat surfaces which were found to range between 14 and 30 m^2 (median 20 m^2). Between 0.09 and 0.27 litres were applied per square metre (median 0.22 l m^{-2}) at a work rate between 2.3 and 7.5 minutes per square metre (median 4.4 min m^{-2}).

The Environment Agency report has some comparative data on active substance and product usage. It indicated that the average boat size was around 30 ft, with motor boats (about 25 % of all boats) generally larger than sailing boats (about 75 % of all boats). Fouling was removed by pressure washing and exhausted antifoulant removed with abrasive and/or a stripping preparation. Most boat owners applied antifouling themselves using a paint roller, annually. Around 15 % of owners applied antifouling less than annually. The quantities applied ranged from 0.1 to 0.3 litres/square foot of boat. Antifouling took place mostly on hard standing or in a boat park. Chandlers were found generally to sell more than 23 products: 163 chandlers belonged to the British Marine Industries Federation, accounting for about 70 % of all chandlers. Antifoulant was sold mostly between February and May.

Amateurs normally apply antifouling products over one day or two consecutive days, normally during fine weather. The application time was found in the HSE survey to range between 35 and 112 minutes (median 90 minutes).

4.2.4.2 Work Clothing

Work clothing worn by amateurs provides some degree of protection. While clothing will tend to retain liquids and dusts to some extent, there will also be run-off. Amateurs were found normally to wear coveralls and cloth gloves for painting. However, anecdotal evidence suggests that antifoulant application wearing minimal clothing is not uncommon. It is expected that chandlers would wear work clothing and protective gloves.

Certain clothing may be desirable to mitigate general risks to health and safety, such as skin contact with solvent-based products. In such cases, gloves may be specified as a precautionary measure for amateurs to ensure that such general risks are minimised. In addition, certain risk phrases derived through the CHIP [Chemicals (Hazard Information and Packaging for Supply) Regulations] process carry mandatory safety phrases and these will appear on a label even though there may be negligible risk to amateurs using the product.

While amateurs may wear clothing such as coveralls or a long-sleeved shirt and long trousers to apply non-agricultural pesticides, this cannot be assured, so may not be assumed for risk assessment purposes. Where there is no better information, default values for penetration to the skin are proposed at 5 % when wearing clothes that provide some degree of protection (professionals at all times) and at 50 % for the realistic worst cases i.e. when minimal clothing is worn (by amateurs). The realistic worst case for amateurs also assumes that no gloves are worn.

4.2.4.3 Exposure Data For Brush And Roller Application

Detailed calculations are presented in Appendix 1, Tables A1.8 to A1.10. The very sparse exposure data relate to 9 amateurs painting their own vessels, with the boat on a cradle, trailer or sling. All but one of the painting jobs were outdoors, with both brush and roller used in most cases. There were 9 data for potential dermal exposure, 7 data for exposure inside gloves and 2 data for exposure to bare hands. Of the 9 subjects, only 4 showed any exposure by inhalation. About half of the potential dermal exposure was to the legs.

4.2.5 SYSTEMIC EXPOSURE DURING BRUSH, ROLLER AND SPREADER APPLICATIONS

4.2.5.1 Calculations

The data sets and exposure calculations are set out in Appendix 1.

Assumptions :

- 10 % active substance in the product
- 10 % dermal penetration default.
- 5 % clothing penetration

Table 4.10 : Contact And Systemic Exposure To Dichlofluanid For Amateurs Users - Application By Brush And Roller - 5 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[1.5 hour job; 5 % central tendency clothing penetration, 50 % worst case - minimal clothing; 1020 mg h^{-1} central tendency, 6480 mg h^{-1} 95th percentile; 31 (median) & 4400 (worst case - no gloves) mg h^{-1} of product on hand]*	123	11500
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	12.3	1150
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**1.2**	**115**
Intake of product by inhalation (mg d^{-1}) *[1.5 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.04 mg m^{-3}, realistic worst case 0.11 mg m^{-3} of product]*	0.04	0.21
In air concentration of dichlofluanid without RPE mg m^{-3}	0.002	0.011
Amount of dichlofluanid inhaled (mg d^{-1})	**0.004**	**0.02**
Total systemic exposure for 60 kg person (mg kg^{-1} d^{-1})	**0.02**	**1.9**

Table 4.11 : Contact And Systemic Exposure To Dichlofluanid For Professionals (Chandlers) - Application By Brush And Roller - 5 % Clothing Penetration

exposure item	central tendency	worst case
Amount of product in contact with skin (mg d^{-1}) *[1.5 hour job; 5 % clothing penetration; 1020 mg h^{-1} central tendency, 6480 mg h^{-1} 95th percentile; 31 (median) & 1100 (worst case) mg h^{-1} of product in-glove]*	123	2160
Percentage of active substance in product (%)	10	10
Amount of dichlofluanid in contact with skin (mg d^{-1})	12.3	216
Dermal absorption value (%)	10	10
Systemic exposure to dichlofluanid via dermal route (mg d^{-1})	**1.2**	**21.6**
Intake of product by inhalation (mg d^{-1}) *[1.5 hour job, 1.25 m^3 h^{-1} inhaled volume, central tendency 0.04 mg m^{-3}, realistic worst case 0.11 mg m^{-3}, of product]*	0.04	0.21
In air concentration of dichlofluanid without RPE mg m^{-3}	0.002	0.011
Amount of dichlofluanid inhaled (mg d^{-1})	**0.004**	**0.02**
Total systemic exposure for 60 kg operator (mg kg^{-1} d^{-1})	**0.02**	**0.36**

4.2.5.2 Assessment Of Risks During Application By Brush, Roller And Spreader

The levels of exposure estimated for professional and amateur users have been used to calculate the toxicity:exposure ratios (TERs) set out in Table 4.12.

Table 4.12 : TERs For Professional And Amateur Users During Application By Brush And Roller

user	central	TER	worst case	TER

	tendency exposure (mg kg^{-1} d^{-1})		exposure (mg kg^{-1} d^{-1})	
professional user (NOEL 2.5 mg kg^{-1} d^{-1})				
5 % clothing penetration				
chandler	0.02	125	0.36	7
amateur user (NOEL 2.5 mg kg^{-1} d^{-1})				
5 and 50 % clothing penetration				
	0.02	125	1.9	1
inhalation exposure (LC_{50} 1223-1338 mg m^{-3})				
professional and amateur	0.002 mg m^{-3}	~600,000	0.011 mg m^{-3}	~100,000

4.2.5.2.1 Professional Users

The TERs for inhalation exposure and central tendency systemic exposure are acceptable but the TER for worst case systemic exposure is of some concern. However these values are derived using a default skin penetration value of 10%. In addition, users may only be exposed to dichlofluanid infrequently, given the typical patterns of use for these products. The ACP therefore considered that the risk assessment was conservative and that uses could continue provided that data on the dermal penetration of dichlofluanid from formulations representative of approved antifouling products were generated in order to refine the risk assessment. The PPE to be worn by professional users is detailed in Section 9 (Recommendations And Data Requirements).

Approvals have been granted for antifouling products containing dichlofluanid for application by spreader by amateurs and professionals. HSE has no data on application of antifouling products by spreader. However, it is possible that painting and spreading take place consecutively. Exposure in spreading is likely to be similar to that in painting - principally to the hands, with the legs receiving the greatest proportion of exposure thereafter, and exposure by inhalation, minimal. There is no information on the typical duration of a spreading job, but it is unlikely to exceed that for painting by a large margin. Consequently, application by spreader is considered acceptable; users to wear the PPE detailed in Section 9.

4.2.5.2.2 Amateur Users

Systemic and inhalation exposure of amateurs applying antifouling by brush or roller is considered acceptable. The ACP, during its consideration of skin sensitisation of substances used in antifouling products (see section 4.1.1), concluded that amateur application by roller brush or spreader could continue where there were no, or only very rare, reports of skin sensitisation or severe irritation in humans and where the use of the active ingredient or coformulant (eg rosin) had been fairly widespread.

In applying these criteria to dichlofluanid, the ACP noted that animal data showed that dichlofluanid should be classified as a skin sensitiser but there were very few reports of skin sensitisation in humans. In addition the ACP noted that there were no reports of skin

sensitisation in workers involved in the manufacture of dichlofluanid. Consequently it considered that amateur application by roller brush or spreader could continue with the recommendation that users where gloves as a precautionary measure.

4.2.6 EXPOSURE AND RISK ASSESSMENT FOR BYSTANDERS

No data are available for consumer exposure to antifouling products. It is considered that bystander exposure to antifouling products applied to the hull of large commercial vessels will be lower than for other workers, since spraying operations are generally avoided by others in the dry-dock, vessels do not emit dichlofluanid once antifoulant has settled on the ship surface and contact with wet surfaces by third parties is unlikely. The risk of skin sensitisation for bystanders is considered minimal.

For amateur applications, passers-by within a congested yard could contact the hulls of freshly treated boats. There is a very low risk of skin sensitisation for bystanders if such exposure were repeated.

4.3 CONDITIONS AND DATA REQUIREMENTS :

1. `Professional use by brush, roller, spray, spreader and aerosol (containing up to 1.5% of dichlofluanid may continue.

2. All professional operators exposed to antifouling products containing dichlofluanid should wear a disposable coverall with hood (providing head protection) and a second overall beneath this coverall of a contrasting colour to the antifouling product being applied. All bare skin should be covered. The disposable coverall should normally be used for no more than one spraying session. The second overall should be changed regularly and whenever product breakthrough has been detected.

3. Professional operators working with dichlofluanid-containing antifouling products should wear impermeable gloves of a type recommended by the antifouling manufacturer as suitable for use with the formulation. These gloves should be changed regularly, e.g. after one or two days' use. Operators should wear impermeable (and non-slip) footwear that protects the lower leg.

4. Professional operators (sprayers) exposed to antifouling products containing dichlofluanid must wear RPE. Appropriate RPE includes air-fed respiratory equipment with combined protective helmet and visor to protect the skin of the head and neck. Impairment of vision should be avoided. For non-sprayers, the need for RPE should be informed by a COSHH assessment.

5. The following approval conditions are to appear on professional-use products' Notices of Approval and Schedules and they should be reflected on product labels using the following precautionary phrases :

WEAR SUITABLE PROTECTIVE CLOTHING (COVERALLS OF A CONTRASTING COLOUR TO THE PRODUCT BEING APPLIED, BENEATH A DISPOSABLE COVERALL WITH HOOD), SUITABLE GLOVES, AND IMPERVIOUS FOOTWEAR THAT PROTECTS THE LOWER LEG.

DISPOSE OF PROTECTIVE GLOVES AFTER USE

If the product is to be applied by spray :

WEAR SUITABLE RESPIRATORY EQUIPMENT SUCH AS AIR-FED RESPIRATORY EQUIPMENT WITH COMBINED PROTECTIVE HELMET AND VISOR WHEN SPRAYING.

DO NOT BREATHE SPRAY MIST.

6. The potential for skin sensitisation and possibly for respiratory irritation as a result of the amateur use of antifouling products containing up to 10 % w/w dichlofluanid is sufficiently low that approval for application by brush, roller and spreader, subject to the fulfillment of data requirements, should be allowed to continue. However, as a precautionary measure, users should wear gloves.

7. As the potential for dermal exposure during spraying or aerosol application is much greater than during application by brush, roller or spreader, approvals for amateur application by spraying be revoked.

8. Data Requirements :

Approval Holders for antifouling products containing dichlofluanid should submit a study of the dermal penetration of dichlofluanid from formulations representative of approved antifouling products. This study may be carried out *in vivo* or *in vitro*;

5. ENVIRONMENTAL FATE AND BEHAVIOUR.

5.1 HYDROLYSIS

In 1982, a 30 d study was carried out to assess the hydrolysis of dichlofluanid (99 %) at pH 4, 7 and 9. The study was reported to follow OECD guideline No 111, but not GLP.

Aqueous solutions of 1 mg l^{-1} dichlofluanid were prepared at pH 4, 7 and 9 using the appropriate buffer solutions, redistilled water and 1 % acetonitrile as a co-solvent. At regular intervals samples were removed and analysed using high performance liquid chromatography (HPLC).

At pH 9 the hydrolytic degradation of dichlofluanid was reported to be so rapid that at room temperature (actual temperature not quoted)**,** even when HPLC analysis was carried out immediately, no dichlofluanid could be detected. The calculated half-lives for the various temperatures at pH 4 and 7 are shown in Table 5.1.

Table 5.1 : Hydrolysis Half-lives Of Dichlofluanid

buffer	temperature	half-life (h)
pH 7	20 ^{o}C	25.6
pH 7	30 ^{o}C	5.4
pH 4	30 ^{o}C	165.6
pH 4	40 ^{o}C	67.2

The half-lives at 22 ^{o}C were determined graphically, via extrapolation, to be < 10 min, 18.8 h and 15.3 d at pH 9, 7 and 4 respectively. It was not stated why 22 ^{o}C was the temperature chosen for the extrapolation. Degradation of dichlofluanid via hydrolysis increased with increasing alkalinity. Quantities of metabolites were not reported in the study.

During further testing, the hydrolysis of the major hydrolytic product dimethylaminosulfanilide was assessed at pH 4, 7, and 9. Dimethylaminosulfanilide samples between 8 and 10 mg l^{-1} (the number of samples and the actual concentrations tested were not reported) were incubated for a period of 1 week at either 55 ^{o}C or kept in a refrigerator (the exact temperature was not stated). No degradation was observed in the dimethylaminosulfanilide samples incubated at 55 ^{o}C when compared to refrigerated samples. The result corresponded to a half-life of greater than one year for dimethylaminosulfanilide at 22 ^{o}C for all pH's. [Unpublished, 1982]

5.2 ACTIVATED SLUDGE, RESPIRATION INHIBITION

In 1987 a study was conducted to investigate the toxicity of dichlofluanid to activated sludge bacteria. The study was conducted to OECD guideline 209. It was not stated whether the test was conducted to GLP. Only a summary sheet of the study was submitted.

Activated sludge bacteria were exposed to nominal test concentrations of dichlofluanid of 1.0, 1.8, 3.2, 5.6 and 10.0 mg l^{-1}. Two control vessels were also established, one containing inoculum and water alone and the second containing only dichlofluanid and water; the latter vessel being the chemical oxygen demand (COD) measurement. A reference substance, 3,5-dichlorophenol, was tested at concentrations of 1.0 and 20.0 mg l^{-1}. It was not reported whether tests were replicated. All vessels were maintained at pH 8 and 20 ± 1 ^{o}C. Results are presented in Table 5.2.

Table 5.2 : Respiration Rates And Percentage Inhibition For Activated Sewage Sludge Exposed To Dichlofluanid

	concentration (mg ai l^{-1})				
	1.0	**1.8**	**3.2**	**5.6**	**10.0**
respiration rate (mg l^{-1} per hour)	42	42	38	32	16
% inhibition	0	0	0	11	55

Inhibition in the respiration rate of sludge micro-organisms was reported at ≥ 5.6 mg l^{-1} dichlofluanid. The auto-oxidation vessel indicated no signs of respiration confirming that dichlofluanid did not oxidise without the aid of micro-organisms.

The EC_{50} value of 9.42 mg l^{-1} for dichlofluanid was calculated by probit analysis (CL8.20 - 11.72). [Unpublished, 1987(a)]

5.3 AQUATIC DEGRADATION

No company-generated aquatic degradation studies were submitted. However, a published study by Callow and Finlay (1995) reported a bioassay method to measure the degradation of dichlofluanid and a number of other antifouling biocides. The test measured degradation by bioassay using *Amphora coffeaeformis* (a marine diatom). The test concentration was fixed by the response of the test organism to dichlofluanid. A concentration between the EC_{50} and EC_{90} was required, with the preferred concentration being the EC_{80} (the EC_{80} allows the loss of biocidal activity over a period of time to be clearly demonstrated).

Degradation studies were performed for a period of 6 - 8 weeks. Vials were prepared containing 9.5 ml of sterile seawater (autoclaved natural seawater). At each time interval six replicates were dosed with 10 µl dichlofluanid at a concentration equivalent to the EC_{80}; vials were then capped and stored in the dark at 25 ^{o}C. This procedure was repeated at 1 - 2 week intervals throughout the test to give samples aged for varying lengths of time. Controls were dosed with 10 µl DMF at the longest (8 or 6 w) and the shortest (0 w) time intervals. Three replicates of each treatment were used for bioassay counts.

At 0 w, 0.5 ml of concentrated *A. coffeaeformis* culture (corresponding to a chlorophyll *a* concentration of 0.25 µg ml^{-1}) was added to each vial containing 9.5 ml of seawater. Dichlofluanid in DMF (10 µl) at a 1000-fold final concentration was added to each vial at an appropriate range of concentrations. Controls contained 10 µl DMF. Each test was replicated three times. Vials were incubated on an illuminated orbital shaker at 150 rpm and at 20 ^{o}C for

96 h. Growth was measured as levels of chlorophyll *a,* after extraction with dimethyl sulphoxide (DMSO). The concentration of the biocide corresponding to the EC_{80} was determined from the plot of chlorophyll *a* versus concentration of biocide. The EC_{80} for dichlofluanid was 5.0 mg l^{-1}. Under the conditions of the test degradation was complete by 72 h, with the half-life calculated as 18 h.

5.4 DEGRADATION IN SOIL

5.4.1 AEROBIC METABOLISM

In 1975 a study was carried out to investigate the degradation of a dichlofluanid formulation (99 %) in two soil types (soil I - organic carbon 2.58 % and pH 6.8; soil II - organic carbon 1 % and pH 5.2). The study was reported to have been conducted to a 1973 Biologische Bundesanstalt fur Land und Forstwirtschaft Guidelines [BBA Guideline Leaflet No.36]. Only a summary sheet of the study (translation sheet) was submitted for evaluation, therefore information on test conditions and procedures was limited.

Soils were treated with 1 mg of dichlofluanid formulation per 100 g test sample; no further test methodology was given. Soils were sampled on days 0, 4, 7, 14, 25 and 48, and analysed for dichlofluanid and dimethylaminosulfanilide using gas chromatography (GC) with a thermionic detector (TID).

Dichlofluanid was degraded in both soils; the process was quicker in soil I than soil II. The levels of dichlofluanid remaining in both soils by study termination reached ≤ 0.5 % AR. In both test soils dichlofluanid was degraded to the major metabolite dimethylaminosulfanilide (specific amounts were not given). The half-life for dichlofluanid was determined to be approximately 2 d for soil I and 3 d for soil II. [Unpublished, 1975]

A study was carried out (no date was given) to investigate the degradation of dichlofluanid (99 %) in two loamy sand soils with a high and moderate humus content (pH 5.5 - 7.5, organically bound carbon 2.5 and 1 % respectively). The study was reported to have been conducted to BBA Guidelines; GLP was not reported. However, only a summary of the results was submitted for evaluation.

Soil samples (50 g dry weight) were placed in eight flasks, moistened and kept for 14 d at 22 ± 2 °C. After this period, 200 µl of [^{14}C]-dichlofluanid dispersed in water (equivalent to an application rate of 10 mg kg^{-1}) was added to each flask, and the contents mixed thoroughly. Flasks were then stoppered, connected to traps to collect evolved $^{14}CO_2$ and incubated in the dark at 22 °C.

Approximately every 2 - 3 d, traps were removed and aliquots of the solution were analysed by liquid scintillation counting (LSC). Soils were sampled at 3, 7, 14, 31 and 63 d after application of dichlofluanid. The radioactivity of the non-extractable fraction was determined by combustion analysis. The half-life for the formation of $^{14}CO_2$ (mineralisation) and for the extractable radioactivity are summarised in Table 5.3.

Table 5.3 : Half-lives Of Dichlofluanid Relative To $^{14}CO_2$ Formed And Extractable Radioactivity

sampling day	soil I (highly humus)		soil II (moderately humus)	
	% $^{14}CO_2$ formed	% extractable ^{14}C-activity	% $^{14}CO_2$ formed	% extractable ^{14}C-activity
3	45	4.7	36.4	57
7	61.5	1.3	57.2	5.3
14	69.5	0.92	77.1	20.4
31	74	0.52	92.7	1.7
63	77.5	0.36	98.5	0.8

Soil I underwent 50 % mineralisation to CO_2 within the first 3 - 4 d post treatment. At day 14, 70 % of the radioactivity was converted to CO_2; in total 78 % was mineralised by day 63. Soil II was found to have undergone 50 % mineralisation by day 5, with 77 % of dichlofluanid converted to $^{14}CO_2$ after 14 d and 98 % after 63 d. [Unpublished, undated]

In 1986 a study was carried out to investigate the metabolism of [phenyl-ring-UL-^{14}C] dichlofluanid (99 %) under aerobic conditions in a sand soil (Type I) and in two sandy loam soils (Type II and Type III) - see Table 5.4 for soil characteristics. In addition, metabolism of radiolabelled-dichlofluanid was investigated using sterilised Type II soil (sandy loam). The study was conducted to BBA guidelines [leaflet No.56] and EPA FIFRA Guideline No. 162-1, but not to GLP.

Table 5.4 : Test Soil Characteristics

soil type	classification	organic carbon content (%)	pH	T_0 biomass (mg C kg^{-1} dry soil)
type I	sandy soil	0.8	5.4	90
type II	sandy loam	2.6	7.1	340
type III	sandy loam	1.3	5.2	243
sterile Type II	sandy loam	2.6	7.1	0

T_0 - test initiation time.

Soil samples (100 g dry weight) were taken from a parent batch treated with [phenylring-UL-^{14}C] dichlofluanid to give an average dichlofluanid concentration of 1 mg kg^{-1} of soil.

During the test, incubation vessels were stored in the dark at 22 ± 3 °C and with a relative humidity of 60 - 80 %. Water was added periodically to maintain the soils at the required moisture content and traps for $^{14}CO_2$ and other volatile compounds were connected to the incubation vessels.
Soils were sampled at various intervals over the study periods. Samples were extracted with solvents and analysed by TLC. Radioactivity in the extracted fraction was determined by ashing (in an automatic oxidiser), and the non-extractable fraction (bound residues) by ashing

and LSC**.** The parent compound and degradation products were identified by thin layer chromatography (TLC) and mass spectroscopy (MS) through comparison with reference standards. Measurements of biomass were also made periodically throughout the test.

With the exception of the sterile soil sample the parent compound was rapidly degraded in all soils to the major metabolite dimethylaminosulfanilide. The half-life of dichlofluanid was determined to be less than one day. After 90 d the percentage of parent compound was less than 0.1 % in the active soils, while 53.2 % was still present in the sterile soil.

In addition to dimethylaminosulfanilide, a further metabolite, methlylaminosulfanilide was identified. This metabolite reached its highest concentration (8.2 % AR) in soil Type II after 97 days. Of the AR ≤ 4.2 % could not be identified.

Mineralisation was highest in the soil Type III with a mean value of 20.4 % after 181 d. In the course of the degradation of dichlofluanid, bound residues (amount not quoted) occurred from which dimethylaminosulfanilide and small amounts of methylaminosulfanilide could be identified. By day 97, 44.8 and 61.6 % of the applied radioactivity was bound to the Type II soil and Type III soil respectively. [Unpublished, 1988]

5.4.2 ANAEROBIC METABOLISM

In 1987 a study was carried out to investigate the metabolism of [Phenyl-ring-UL-^{14}C] dichlofluanid (99 %) under anaerobic conditions in a sandy loam (Type III soil used previously for the aerobic studies). In a separate experiment the soil was pre incubated for 30 d under aerobic conditions prior to the anaerobic phase. The study was conducted in accordance with Protocol M 1260103-3 (no further information was provided), and the aerobic pre-incubation phase to EPA FIFRA Guideline No.162-2, but not to GLP.

Soil samples (100 g dry weight), treated with dichlofluanid to give a mean measured concentration of 0.87 mg kg^{-1} of soil, were placed in ten incubation vessels. Six of the vessels were placed immediately under anaerobic conditions. These flasks were then flushed out with nitrogen, closed, and stored in the dark at 22 ± 2 ^{o}C and 60 - 80 % relative humidity. Duplicate soil samples were removed for analysis following 30, 60 and 90 d periods of anaerobic incubation.

In the remaining four incubation vessels, soil moisture content was adjusted and traps for $^{14}CO_2$ and other volatile compounds were connected to the vessels. Following an aerobic pre-incubation period of 30 d, these vessels were flushed with air, traps were removed and vessels were introduced to anaerobic conditions. At 31 and 60 d post treatment, duplicate soil samples were removed for analysis.

Following incubation, the head space of the vessel was analyses for $^{14}CO_2$ and $^{14}CH_4$. The surface water and soil were analysed separately for radioactivity by a combination of ashing and LSC. Parent compound and metabolites were identified by TLC.
The parent compound was rapidly degraded under anaerobic conditions to the major metabolite dimethylaminosulfanilide (≤ 23.3 % AR). Small amounts of methyl aminosulfanilide (≤ 0.2 % AR) were also detected. Dichlofluanid was not detected (< 0.1 %) on any of the sampling days.

At 90 days, ≤ 0.3 % AR $^{14}CO_2$ was formed under purely anaerobic conditions. After 30 days under aerobic conditions, mineralisation accounted for 4.1 % of the applied radioactivity (mean value). The mineralisation ceased when the aerobic system was switched to anaerobic conditions. Methane did not occur as a degradation product (< 0.1 % AR). The proportion of bound residues was distinctly lower in the anaerobic only systems (10.8 % AR) compared to those with aerobic pre incubation (59.3 % AR). The study authors postulated that aerobic processes and/or aerobes were presumed to have incorporated the metabolites into the soil matrix. The anaerobic half-life was not calculated. [Unpublished, 1987(a)]

Figure 5.1 gives the degradation pathway of dichlofluanid.

5.5 MOBILITY IN SOIL

In 1985 a soil column study was carried out to determine the leaching behaviour of [U-^{14}C]phenyl-dichlofluanid (99 %) aged in soil. The study was conducted to Bulletin No.37 of the Biologische Bundesanstalt fur Land und Forstwirtschaft Guidelines (BBA), but no indication was given as to whether it was carried out to GLP.

Samples (100 g dry weight) of a BBA standard soil (organic content 0.69 %, pH 7.0) were treated with a mixture of labelled and unlabelled dichlofluanid, placed in incubation vessels and the water content of the soil was adjusted. The application used was based on the maximum rate used in practice (2.5 kg active ingredient ha^{-1}), which corresponded to 0.5 mg per column. Of the eight test samples, two were analysed immediately for determination of the initial radioactivity in the soil. A further two samples were subjected to leaching without ageing (day 0), and remaining duplicate samples were aged for a period of either 30 or 90 d. During ageing, samples were stored in the dark at a temperature of 22 ± 2 ^{o}C and relative humidity (RH) of 60 - 80 %. Traps for collecting volatiles and $^{14}CO_2$ were connected to the incubation vessels.

Six soil columns, 26 cm in length (diameter measurement was not quoted) were prepared using the standard soil. Following saturation of the column with water, duplicate soil samples incubated with dichlofluanid (aged for either 0, 30 or 90 d) were placed in a layer on top of each soil column and water was added (the volume of water added was not stated).

Following percolation, the leachate was collected in two fractions of 200 ml and analysed by TLC and LSC. Soil columns were frozen, extruded from the glass columns and divided into three equal sections. The radioactivity in each section was determined by combustion analysis.

The radioactivity recovered corresponded to the quantity applied. Comparison of the initial radioactivity with that after the period of ageing indicated that considerable quantities of $^{14}CO_2$ were released during ageing. Only a small proportion of the applied radioactivity was actually put on the soil column (the amount was not reported) for the watering procedure (at day 90).

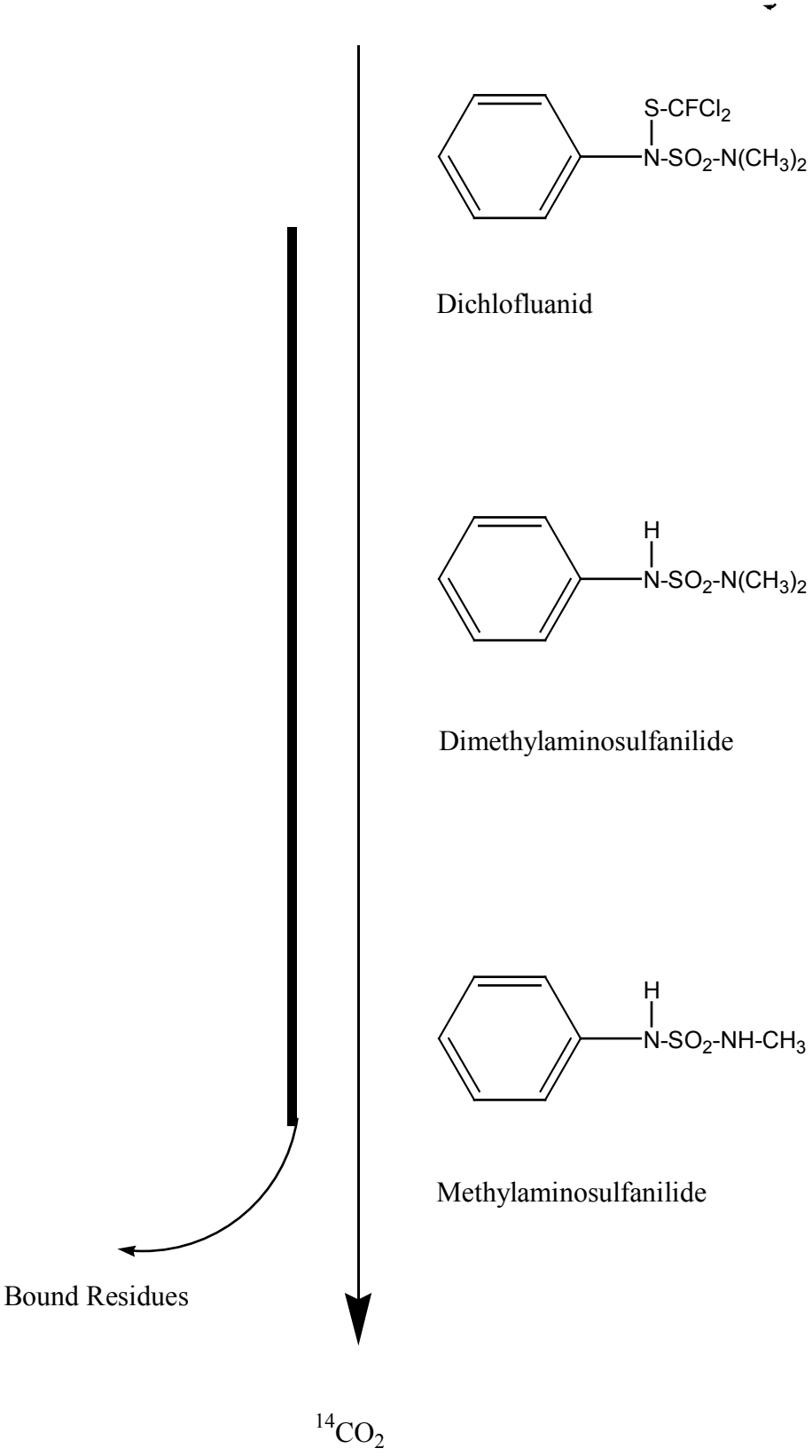


Figure 5.1 : Proposed Degradation Pathway Of Dichlofluanid In Soil

After the aged residues (day 90) had been watered, about 80 % of the radioactivity put into the soil column was found to have remained in the upper third of the column.

In the 0 and 30 d samples, the leachate contained 65.5 % of the recovered radioactivity. After 90 d of ageing, this was found to have declined to a level of 3 %. In the leachate less than 1 % of the radioactivity was parent compound. The majority of the radioactivity consisting of the metabolite dimethylaminosulfanilide (62 - 66 %) in the 0- and 30 d samples, and an unidentified metabolite in the 90 d sample.

Under the conditions of the test, the study authors proposed that dichlofluanid qualified for classification as immobile, and the major metabolite dimethylaminosulfanilide was considered to be mobile. This metabolite was no longer present in the leachate (<1 %) after 90 d of ageing. [Unpublished, 1985]

A further study was carried out in 1987 to determine the leaching behaviour of [phenyl-UL-^{14}C]-dichlofluanid (99 %) and [phenyl-UL-^{14}C]-dichlofluanid formulation [99 % wettable powder (WP)]. A soil column method was used with two different modes of application (with and without incorporation into the soil). The study was conducted to Bulletin No.37 of the Federal German Biological Research Agency (BBA), but not to GLP.

The study was performed using a standard Type I soil (same soil as that used in the previous soil column leaching study). The active ingredient or formulation was applied to soil (100 g dry weight) which was then spread in a layer on the top of a leaching column. In addition, the formulation was also applied dropwise onto the surface of the leaching column having first been suspended in 1 ml of water. Both applications achieved a concentration of 0.49 - 0.5 mg of active ingredient per column.

Duplicate soil columns were prepared for each test. Each column was 26 - 30 cm long and 5 cm in diameter. Prior to the addition of the test material each column had been saturated with water and was then watered with approximately 400 ml of water over a 48 h period.

The leachate was collected in two fractions of 200 ml and analysed by TLC and LSC. Soil columns were frozen, extruded from the glass columns, and divided into three equal sections. The radioactivity of each section was determined by combustion analysis. The parallel experiments (leachates) were in good agreement. Consequently, only one of the replicates (the one with the higher level of radioactivity in the leachate) was analysed for each test. The results are shown in Table 5.5.

Table 5.5 : Leaching Of ^{14}C-labelled Dichlofluanid In Soil

	percentage of applied radioactivity					
	WP formulation applied dropwise		WP formulation incorporated into soil		unformulated ai incorporated into soil	
replicate	**A**	**B**	**A**	**B**	**A**	**B**
1. soil						
upper third	-	78.9	-	52.2	16.3	-
middle third	-	10.6	-	14.5	23.3	-
lower third	-	5.1	-	13.7	28.2	-
2. leachate						
total	0.4	1.3	7.6	11.5	32.4	28.1
fraction I	<0.001	0.01	<0.1	0.2	0.1	<0.1
fraction II	0.4	1.3	7.6	11.3	32.3	28.1
total	-	95.9	-	91.9	100.2	-

The overall recovery of radioactivity was good. After dropwise application of the suspended WP formulation to the soil surface, the leachate was found to contain very little of the radioactivity originally applied (0.4 and 1.3 %). After incorporation of the formulation into the soil, the fraction of radioactivity in the leachate increased (7.6 and 11.5 %). Incorporation of the unformulated active ingredient into the soil resulted in the highest fraction of radioactivity in the leachate (28.1 and 32.4 %).

Analysis of the leachate by TLC determined that fraction II contained < 0.1 % of the parent compound with both the active ingredient or formulation. However, it contained the metabolite dimethylaminosulfanilide (respectively 32 % and 10 % of the applied radioactivity). Characterisation of the leachate from dropwise application of the formulation was not performed.

The results indicated that the leaching behaviour of the active ingredient was dependent on the form in which the dichlofluanid was applied (i.e. formulation or unformulated dichlofluanid). The unformulated dichlofluanid was found to be more mobile than the formulated dichlofluanid. This was presumed by the study authors to be connected with the rapid biotransformation of the active ingredient into a mobile metabolite. Under the conditions of the test dichlofluanid was classified, based on the Helling and Turner classification scheme, as immobile regardless of the method of application. The metabolite dimethylaminosulfanilide was classified as immobile to slightly mobile. [Unpublished, 1987(b)]

5.6 BIOCONCENTRATION

In 1991 a study was carried out to determine the bioconcentration and elimination of dichlofluanid (99 %) by bluegill sunfish (*Lepomis macrochirus*). The study was reported to follow EPA FIFRA guidelines and ASTM guidelines, and was conducted to GLP.

One group of 56 sunfish were continuously exposed to a mean measured concentration of 4.4 (± 0.2) µg l^{-1} ^{14}C-dichlofluanid (99 %) dissolved in acetone for 28 d. A solvent-control group was also used. At the end of this period, aquaria were cleaned, emptied and filled with uncontaminated water. The fish were then exposed to continuous flowing uncontaminated diluent water for a depuration period of 14 d. Throughout the study, the temperature was maintained at 22 ± 1 ^{o}C; dissolved oxygen levels ranged between 83-109 % saturation and the pH ranged from 6.9 to 7.7. Fish and water were sampled for ^{14}C-compounds on days 0, 1, 3, 8, 10, 14, 21 and 28 (exposure period) and on days 29, 31, 35, 38 and 42 (depuration period). Four fish were removed at each time interval and the ^{14}C-residues in the edible, non-edible and whole body tissue determined by radiometric analysis (LSC).

From the results generated, a mean steady state BCF was calculated for each of the edible, non-edible and whole fish portions on each sampling date. The uptake constant (K_1) and depuration rate constant (K_2) were determined. The results are shown in Table 5.6.

Table 5.6 : Mean (± Standard Deviation) Dichlofluanid Bioconcentration And Elimination Data

tissue	BCF at steady state	time to reach 90 % of steady state (d)	depuration $T^1/_2$ (d)
edible (muscle tissue)	61 (±9)	0.82 (±0.09)	0.25 (±0.03)
non-edible (viscera and carcass)	87 (±13)	1.27 (±0.14)	0.38 (±0.04)
whole fish	72 (±14)	0.80 (±0.11)	0.24 (±0.03)

During the 42 d study no mortalities were recorded and fish appeared healthy and behaved normally. After 24 h exposure to uncontaminated water, 84, 86 and 85 % of the maximum measured plateau residues were depurated from edible, non-edible and whole fish respectively. After 7 d in uncontaminated water, > 99 % of ^{14}C-residues had been eliminated from edible, non-edible and whole fish portions.

Dichlofluanid bioconcentrated in the bluegill sunfish with a total residue bioconcentration factor of 73 for whole fish. After exposure ceased, residues were depurated quickly with a half-life of < 6 h. [Unpublished, 1991]

5.7 SUMMARY OF ENVIRONMENTAL FATE AND BEHAVIOUR DATA

The studies submitted were all conducted to internationally accepted guidelines; GLP compliance has been addressed with each individual study.

A 30 d hydrolysis study resulted in a half-life of 25.6 h for dichlofluanid (99 %) at pH 7, 20 ^{o}C. At pH 9 dichlofluanid hydrolysed so rapidly that no parent compound could be detected. A microbial inhibition study resulted in an EC_{50} of 9.42 mg ai l^{-1}. However, this result should be treated with caution as the validity of the test was unclear. Biodegradation of dichlofluanid

in seawater was investigated by a published study using a bioassay technique and reported a half-life of 18 h. The degradation of dichlofluanid in soil was investigated in a number of studies. Aerobic degradation studies resulted in half-lives of 2 - 5 d for dichlofluanid depending on soil type and humus content, with dichlofluanid being degraded to the primary metabolite dimethylaminosulfanilide and smaller amounts of methylaminosulfanilide. Anaerobic degradation studies confirmed that dichlofluanid was rapidly degraded to the primary metabolite dimethylaminosulfanilide. Dichlofluanid was not detected on any sampling days after test initiation, and no half-life was quoted. Two mobility studies were performed using leaching columns; both studies confirmed that dichlofluanid was immobile. However, the primary metabolite dimethylaminosulfanilide was classified as mobile in one study and immobile to slightly mobile in the second study. The more robust study indicated that the primary metabolite dimethylaminosulfanilide was more mobile than dichlofluanid and the classification was based on the Helling and Turner classification scheme. Therefore, dimethylaminosulfanilide is considered to be immobile to slightly mobile.

Dichlofluanid has a Log P_{ow} of 3.70 which indicates that there is a potential for dichlofluanid to bioaccumulate. In a bioconcentration study with the bluegill sunfish, dichlofluanid accumulated very rapidly with a total residue bioconcentration factor of 73 for whole fish. However, studies indicated that dichlofluanid depurates quickly with a half-life of < 6 h.

6. ECOTOXICOLOGY

6.1 TOXICITY TO ALGAE

In 1985, a 96 h acute toxicity test was carried out using dichlofluanid (86.2 % dichlofluanid) against the green alga *Scenedesmus subspicatus*. The study was carried out according to OECD guideline No. 201 and was not conducted to GLP.

Following range-finding studies, nominal concentrations of 0.1 and 1.0 mg l^{-1} were prepared in triplicate from a stock solution of dichlofluanid dissolved in dimethylsulfoxide (DMSO). A water-only control and a solvent control were also prepared in triplicate. The effect of dichlofluanid on algal growth was monitored after 24, 48, 72 and 96 h. The temperature during the test was 23 ± 1 ^{o}C. Light intensity was kept at a constant illumination of 8000 lux and pH was monitored throughout the test (pH ranged throughout the test from 7.87 - 8.46).

The guideline suggests that the test should be performed with a range of five concentrations. However, the study authors reported that this was not possible due to the low solubility of dichlofluanid.

In this study both the 96 h E_bC_{50} (biomass) and E_rC_{50} (growth rate) values were found to be > 1 mg l^{-1} (the highest concentration tested). The NOEC could not be established therefore, the study authors assumed that the NOEC would be >1 mg l^{-1}. [Unpublished, 1985]

6.2 TOXICITY TO INVERTEBRATES

6.2.1 ACUTE TOXICITY TO FRESHWATER INVERTEBRATES

In 1986, a 48 h acute toxicity study was carried out using measured concentrations of dichlofluanid (88.5 %) against *Daphnia magna* in a flow-through test system at 20 ± 1 ^{o}C. The test method was reported to follow EPA guidelines and was conducted to GLP.

Following range-finding studies, mean measured concentrations of 0.071, 0.099, 0.24, 0.35 and 1.0 mg l^{-1} were prepared from a stock solution of dichlofluanid dissolved in dimethylformamide (DMF). Four replicates of 10 *Daphnia* were exposed to each concentration, solvent and dilution water controls. The results are presented in Table 6.1.

Table 6.1 : Acute Toxicity Of Dichlofluanid To *Daphnia magna*

parameter	concentration (mg l^{-1}) (with 95 % confidence limits)
24-h EC_{50}	0.57 (0.51-0.67)
48-h EC_{50}	0.42 (0.37-0.47)
48-h NOEC	0.07

Signs of toxicity including surfacing, quiescence and bottom orientation were observed among daphnids at concentrations ≥ 0.099 mg l^{-1}. The 48 h EC_{50} for *Daphnia magna* (with 95 % confidence limits) was reported to be 0.42 mg l^{-1} dichlofluanid. The NOEC, based on lack of mortality and abnormal effects, was reported to be 0.07 mg l^{-1}. [Unpublished, 1986(a)]

6.2.2 CHRONIC TOXICITY TO FRESHWATER INVERTEBRATES

In 1989 a 24 d chronic toxicity study was carried out with dichlofluanid (91.4 %) against *Daphnia magna* under semi-static conditions. The study was carried out to OECD guideline No. 202 part II and was conducted to GLP.

Nominal test concentrations of 0.002, 0.008, 0.04, 0.2 and 1.0 mg 1^{-1} were prepared from a stock solution of dichlofluanid dissolved in acetone. Ten replicates of a single *Daphnia* were exposed to each concentration (1 *Daphnia* in each 50 ml beaker). In addition solvent and water only controls were prepared. The test was carried out at 21.5 - 22.5 ^{o}C and water quality parameters measured throughout the test. The test media was replaced approximately every two days (10 changes in total). The results are presented in Table 6.2.

Table 6.2 : Chronic Toxicity Of Dichlofluanid To *Daphnia magna*

parameter	concentration (mg l^{-1})
24-d NOEC	0.04
24-d LOEC	0.20

No statistically significant effects on the reproduction rate of *Daphnia magna* were observed up to concentrations of 0.04 mg l^{-1}. At a concentration of 0.2 mg l^{-1} a significant inhibition of reproduction was observed (51.7 %) when compared to the controls. Taking into account the reproduction rate, the NOEC was determined to be 0.04 mg l^{-1}. The lowest concentration resulting in significant differences from the control was 0.2 mg l^{-1}.

There were some deviations in the experimentation from the guidelines. Firstly, measured concentrations of dichlofluanid fell consistently below 80 % of normal concentrations throughout the test period. Secondly, rapid hydrolysis of the test substance at a pH > 7 was reported to be the cause of the low analytical results. However based on both these factors, it is stated that calculations of toxicity should have been made using measured concentrations. Since the determined NOEC of 0.04 mg l^{-1} was calculated from nominal concentrations, the result should be treated with caution. The results are likely to be an underestimation of the chronic toxicity of dichlofluanid. The exact toxicity of dichlofluanid cannot be established from this test because the difference at the top two concentrations (0.2 - 1.0 mg l^{-1}) was too large to determine the exact toxicity. [Unpublished, 1989]

6.3 TOXICITY TO FISH

6.3.1 ACUTE TOXICITY TO FRESHWATER FISH

In 1986, 96 h acute toxicity studies were carried out using measured concentrations of a dichlofluanid formulation (88.5 %) against bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. The studies were reported to follow EPA Guidelines (actual guidelines not reported). Both studies were conducted to GLP.

Measured test concentrations were prepared from a stock solution of dichlofluanid dissolved in acetone. Following range-finding studies, 20 bluegill sunfish per group were exposed to dichlofluanid at mean concentrations of 0.50, 0.25, 0.1, 0.05 and 0.024 mg formulation l^{-1} and 20 rainbow trout per group were exposed to mean concentrations of 0.033, 0.016, 0.0066, < 0.0026 and < 0.0026 mg formulation 1^{-1} respectively. Test temperatures were maintained at 22 - 23 ^{o}C for the bluegill sunfish and 12 - 13 ^{o}C for the rainbow trout. Solvent and dilution water controls were included in both studies. The results are shown in Table 6.3.

Table 6.3 : Acute Toxicity Of Dichlofluanid To Fish

parameter	concentration (mg l^{-1}) (with 95 % confidence limits)	
	bluegill sunfish	**rainbow trout**
48-h LC_{50}	[2] 0.031 (0.026-0.037)	[1] 0.010 (0.0066-0.016)
96-h LC_{50}	[1] 0.030 (0.024-0.05)	[1] 0.010 (0.0066-0.016)
NOEC	<0.024	<0.0026

[1] **binomial method**
[2] **moving average method**

Signs of toxicity including loss of equilibrium, orientation at the bottom of the tank, and rapid gill movement were observed in bluegill sunfish exposed to concentrations of 0.10 and 0.05 mg formulation l^{-1} after 48 h. By 96 h, complete mortality had occurred at these two test concentrations. Mortalities were observed at all concentrations tested. Therefore, the NOEC was determined to be < 0.024 mg 1^{-1}. The 96 h LC_{50} (with 95 % confidence limits) was calculated to be 0.030 mg formulation l^{-1} (0.024 - 0.05).

Behavioural responses (e.g. surfacing, orientation at the bottom of the tank and loss of equilibrium) were noted in rainbow trout in the 0.016 and 0.006 mg formulation l^{-1} test levels. One hundred percent mortality was noted at 0.033 and at 0.16 mg formulation l^{-1}, 24 h after test initiation. The NOEC was determined to be < 0.0026 mg l^{-1}. The 96 h LC_{50} was reported to be 0.010 mg l^{-1} (0.0066 - 0.016). [Unpublished, 1986(b) and 1986(c)]

Antychowicz *et al.,* (1979) investigated the effects of dichlofluanid on carp. A 50 % dichlofluanid formulation was diluted to give the following test series : 0.05; 0.15; 0.25; 0.35; 0.4; 0.55; and 0.6 mg l^{-1} formulation. The dichlofluanid induced significant toxic effects in the carp, affecting the central nervous system and the cardiovascular system. Deaths occurred at concentrations between 0.5 - 0.6 mg l^{-1} formulation. Pathological effects were evident on the gill sheets where distal ends became pale due to ischaemia. Areas of ischaemia increased

with increased concentration. The NOEC was determined to be 0.05 mg l^{-1} formulation; the LOEC was determined to be 0.5 mg l^{-1} formulation.

6.4 TOXICITY TO BIRDS

6.4.1 ACUTE ORAL TOXICITY

In 1986, a 14 d acute oral toxicity study was carried out using dichlofluanid (88.5 %) against adult (22 - 24 w old) bobwhite quail (*Colinus virginianus).* The study was carried out to EPA FIFRA guideline 71-1 and conducted to GLP.

All groups consisted of ten birds (five males and five females). One group was given a single oral dose of 2226 mg kg^{-1} bw dichlofluanid in corn oil via oral gavage. Two control groups were given corn oil only. All birds received a dose volume equal to 1 % of their body weight. Food and water were available *ad libitum* throughout the 14 day study period. At study termination post-mortem examinations were performed.

No mortalities or clinical signs of toxicity were noted in the treated quail. Feed consumption and body weight data indicated an initial rejection of feed and a subsequent loss in body weight up until day 7 in treated birds. However, recovery in terms of both feed consumption and body weight was observed by study termination. No dichlofluanid-related gross lesions were noted at post-mortem examination.

The acute oral LD_{50} of dichlofluanid to the bobwhite quail was reported to be > 2226 mg kg^{-1} bw. The NOEC was determined as < 2226 mg kg^{-1} bw. [Unpublished, 1986(d)]

6.4.2 ACUTE DIETARY TOXICITY

In 1986, 8 d acute dietary toxicity tests were carried out using dichlofluanid (88.5 %) against mallard ducks (*Anas platyrhynchos*) and bobwhite quail (*Colinus virginianus*). Both studies were reported to have been conducted to both EPA FIFRA guidelines 71-2 and GLP.

The study consisted of two control groups and one test group. Ten birds per group were used. A single dietary concentration of 5000 ppm, which was corrected for purity, was prepared by mixing the dichlofluanid into the diet with corn oil. The control birds received a corresponding volume of feed and corn oil only. Treated feed and water was presented to the birds over a 5 d exposure period and birds were allowed to feed *ad libitum*. Test conditions were reported to be maintained under a 16:8 h light:dark cycle and at a temperature of 37 ± 1 ^{o}C. After the 5 d period, the birds were fed untreated feed for 3 d. During the study, observations for mortality and signs of toxicity were made twice daily.

In the mallard duck no compound-related mortalities occurred as a result of feeding a diet containing 5000 ppm dichlofluanid for a period of 5 d. Differences in body weights were noted in the treated birds when compared to the controls. However, feed consumption data suggested this was due to feed inpalatability or gastro-intestinal tract irritation. No gross signs of toxicity were noted. Inflamation of the gizzard mucosa was observed at post-mortem

examination in 6 of the 10 ducks fed dichlofluanid; this suggested that the material has a mild irritative effect. No other compound-related lesions were noted.

No compound-related mortalities occurred as a result of feeding a diet containing 5000 ppm of dichlofluanid to bobwhite quail for a period of 5 d. Differences in body weights were noted in treated birds when compared to the controls. However, no differences in feed consumption were observed. No grossly observable signs of toxicity were noted in treated birds throughout the course of the study. No compound-related gross lesions were observed on post mortem examination of quail sacrificed at study termination.

The acute dietary LC_{50} for both species of birds was assumed to be > 5000 ppm. The NOEC was reported as < 5000 ppm. [Unpublished, 1986(e) and 1986(f)]

6.5 SUMMARY OF ECOTOXICOLOGY DATA

The company studies submitted were all conducted to internationally accepted guidelines and to GLP where detailed. The toxicity of dichlofluanid to freshwater algae, invertebrates and fish is summarised in Appendix 2.

Dichlofluanid is toxic to the freshwater algae *Scenedesmus subspicatus* which had 96 h E_bC_{50} and E_rC_{50} values of > 1 mg l^{-1}; this was the highest concentration tested. Dichlofluanid is toxic to *Daphnia magna*, with an acute study resulting in a 48 h EC_{50} value of 0.42 mg ai l^{-1} and a NOEC of 0.07 mg l^{-1}. In a chronic study, *Daphnia* reproduction was inhibited by 51.7 % at 0.2 mg ai l^{-1}. Acute toxicity studies on the rainbow trout and blue gill sunfish resulted in 96 h LC_{50} values of 0.010 and 0.030 mg ai l^{-1} respectively, with NOECs of < 0.024 and < 0.0026 mg l^{-1} for bluegill sunfish and rainbow trout respectively. A study investigating the effects of dichlofluanid on carp resulted in a NOEC of 0.05 mg l^{-1}. Dichlofluanid was found to be of low toxicity to birds following acute oral and dietary tests on the bobwhite quail (LD_{50} > 2226 mg kg^{-1} bw and > 5000 ppm) and the mallard duck (> 5000 ppm).

7. ENVIRONMENTAL RISK ASSESSMENT

7.1 ENVIRONMENTAL HAZARD PROFILE

The leaching rate for dichlofluanid was estimated at 0.6 (ISO/ASTM) and 1.7 (FLUME) μg cm^{-2} d^{-1} using methodologies developed under a research contract for HSE. This active ingredient hydrolysed rapidly at pH 7, with a half-life of only 1.07 d. It was also shown to degrade rapidly in seawater, with a subsequent half-life of 18 h. The major metabolite, dimethylaminosulfanilide, following hydrolysis was shown to be hydrolytically stable. Dichlofluanid was shown to be adsorbed to soil but degraded rapidly, with half-lives of 2 - 5 d reported. Dimethylaminosulfanilide was also shown to be adsorbed to soil but was slightly more mobile than the parent compound. Dichlofluanid did not bioconcentrate in fish, with BCFs of only 61 - 87 and a rapid depuration half-life of less than 6 h. Although no marine data were submitted, dichlofluanid was shown to be highly toxic to aquatic organisms. The species most sensitive to chronic effects was *Daphnia magna*, with a 24 - d NOEC (reduced reproduction) of 40 μg ai l^{-1}.

7.2 RISK ASSESSMENT STRATEGY

The risk assessment has been concentrated on the marine environment since the data available are predominantly for the use of antifouling products (AFPs) in estuarine and coastal areas, although the risk to freshwater environments has not been precluded. However, the strategy for assessing risk to the marine environment is less well developed than for terrestrial or freshwater environments. Therefore, the risk assessment strategy adopted for the current review has been presented in a separate document 'Environmental Risk Assessment of Booster Biocides in Antifouling Products' (ACP 2002). This document presents a comprehensive and comparative risk assessment for all approved booster biocides and has been endorsed by an expert *ad-hoc* Environmental Panel.

For the marine environment, three distinct areas were identified : estuarine (including marinas and harbours); shallow coastal seas; and deep ocean. Within each area considerations of the following compartments : sediment (including suspended sediment); water; and associated biota are required. Therefore the primary objective of this risk assessment has been to establish the likelihood of dichlofluanid reaching (or having reached) a concentration in the aquatic environment which would adversely affect some component of that environment. In order to achieve this objective, measured environmental concentrations (MECs), predicted environmental concentrations (PECs) and predicted no-effect concentrations (PNECs) have been derived.

Below are the main points of the risk assessment detailed in the "Environmental Risk Assessment of Booster Biocides Antifouling Products' concerned with the use of AFPs containing dichlofluanid; however, reference to the complete document is advised.

7.2.1 ENVIRONMENTAL CONCENTRATIONS

The direct and indirect exposure of the aquatic environment to dichlofluanid as a result of AFP use are detailed fully in the Environmental Risk Assessment document Section 3.2. Data have not been made available to address the direct and indirect inputs to the soil and to aquatic compartments as a result of AFP use (application and removal stages). Further to this, HSE have no data regarding the direct exposure of sewage-treatment processes (STP), or the subsequent emissions to surface waters. However, a survey carried out by the Environment Agency in 1998 demonstrated that there was a wide range of use-patterns and work practices regarding the application and removal of AFPs. The survey suggested that emissions via drains would be low since direct exposure of the surrounding water body (marina/harbour) was more likely, with the majority of boat maintenance taking place at the waters' edge. Although the survey did state that where removal by sandblasting was undertaken (usually by boatyards) appropriate waste disposal methods were reported. The contamination of aquatic sediments from direct emissions or via particulate inputs (contaminated soil) is an important route to consider, since the potential to contribute significantly to the environmental loading of the booster biocides exists through remobilisation. Where strongly adsorbed active ingredients are demonstrated, in the aquatic environment mechanical remobilisation or sediment redistribution via dredging are likely. Therefore, exposure scenarios other than direct exposure of the aquatic compartment (i.e. indirect exposure of the aquatic environment, direct exposure of the soil and STP) have not been considered further at this stage as insufficient data is available to fully address the risks. The potential scale of direct exposure to the aquatic compartment may also be considered to outweigh the additional areas of concern at this time.

Usage information was requested from all the current Approval Holders for dichlofluanid. However the information received was too limited to allow for any accurate assessment of the actual amounts used of AFPs containing dichlofluanid. Therefore, the results of a survey conducted by the Environment Agency (EA) in 1998 were used. The EA survey demonstrated that dichlofluanid was currently used on 2.1 - 3.2 % of pleasure-craft in the U.K.. Seawater monitoring data provided by Centre for Environment, Fisheries & Aquaculture Science (CEFAS) on behalf of a Department of Environment Transport and the Regions (DETR) commissioned monitoring program in 1998, demonstrated that dichlofluanid was not detected at any of the sites. No sediment analysis was conducted for dichlofluanid. However the current monitoring data could only ever represent the usage levels for 1998, and predictions of maximum PECs were considered necessary by the *ad-hoc* Environmental Panel since post approval usage cannot be controlled. Therefore, PEC data based on 100 % usage (all vessels treated) of dichlofluanid AFPs were predicted using a model developed as part of a HSE/EA commissioned research project. The model, Regulatory Environmental Modelling of Antifoulants (REMA), is a steady-state quantitative water air sediment interface (QWASI) model, designed to predict concentrations of biocides in both the water and sediment compartments of estuaries/marinas/harbours. The model is based upon for four real estuary scenarios in the U.K., for which the model has been successfully validated. The model inputs used for dichlofluanid are presented in Table 7.1 and a summary of the results is presented in Table 7.2.

Table 7.1 : REMA Model Input Parameters For Dichlofluanid

input	value	source
melting point	105 ºC	company
molecular mass	333.2	company
vapour pressure	3.79 x 10^{-5} Pa	company
solubility in water at 20 ^{0}C	1.3 mg l^{-1}	company
log Koc	3.3	calculated from Kow (company data)
sediment half-life hydrolysis photolysis biodegradation	 10 000 000 h 10 000 000 h 10 000 000 h	 default (no data) default (no data) default (no data)
water half-life hydrolysis photolysis biodegradation	 25.68 h 10 000 000 h 18 h	 company, hydrolysis half-life default (no data) literature study, seawater bioassay
leaching	1.7 µg cm^{-2} d^{-1}	HSE Flume research method
usage (% boats treated)	3.2 % and 100 %	EA 1998 survey and maximum

Table 7.2 : Summary Of PEC Calculations For Dichlofluanid In Estuaries And Open Marinas

usage (%)	site	PECwater (ng ai l^{-1})	PECsediment (mg ai g^{-1})
3.2	estuary*	1.2 (±2.2)	1.5x10^{-6} (±2.4x10^{-6})
	open marina**	17.4 (±0.5)	1.6x10^{-5} (±1.1x10^{-5})
100	estuary*	36.4 (±67.1)	4.6x10^{-5} (±7.5x10^{-5})
	open marina**	543.8 (±327.4)	5.0x10^{-4} (±3.5x10^{-4})

* n = 12, **n = 7

From the above Table, the predictions for concentrations of dichlofluanid in water were greater than the limit of detection quoted by CEFAS (1 ng l^{-1}) even at both usage levels in both marinas and estuary scenarios. The mean PEC calculations for the sediment compartment were very low and, as for chlorothalonil, suggest that regardless of usage or location (marina/estuary) these would be difficult to detect. As yet no information has been supplied by the company which would suggest that an analytical technique has been refined for this active ingredient.

7.2.2 PREDICTED NO EFFECT CONCENTRATIONS

Once released into the aquatic compartment, the chemical fate of the booster biocide will determine whether the toxic effect exerted is limited to the target organisms within a boundary layer of a painted surface or, whether the active ingredient persists and there is potential for exposure to non-target organisms. Therefore, selection of key non-target organisms and likely duration of exposure is essential; however, this is somewhat reliant on the availability of acceptable data for representative marine species. Chronic data endpoints have been selected as more appropriate for the purpose of a marine risk assessment following the use of booster biocides. This is because the inputs of booster biocides into the marine environment as a result of leaching from multiple-point sources (treated surfaces) will be a continuous process. Even where high degradation is indicated from fate studies, the continuous input from leaching can still result in long-term exposure. This will only be mitigated if the leaching rate of an active ingredient from a painted surface is significantly slower than the rate of degradation, and no toxic metabolites are produced.

Comparisons between marine and freshwater chronic toxicity data for booster biocides has not demonstrated any differences in sensitivities. Therefore, in the absence of chronic marine data, freshwater data would be acceptable. Further to this, in considering the number and quality of tests available for the current review, the most sensitive species has been selected, regardless of test medium. However, the introduction of safety factors is required before deriving a PNEC from the available hazard data, which in the absence of additional or more appropriate data will provide a suitable safety margin for all marine organisms. The provision of safety factors will be made in accordance with the guidance detailed in the European Risk Assessment Technical Guidance Document [EURATGD, 1996], and those previously accepted by the ACP. See Appendix 3.

Only freshwater chronic data were available for dichlofluanid. These included algae and *Daphnia magna*; the latter being the most sensitive species for which a 24 d NOEC (reproduction) of 40 μg ai l^{-1} was reported. Therefore, the dichlofluanid toxicity data attracted a safety factor of 50. The PNEC for dichlofluanid for the purposes of this risk assessment is 0.8 μg ai l^{-1} for *Daphnia magna.*

7.2.3 RISK QUOTIENT

The PEC:PNEC calculations derived from the predicted data based on 100 % usage for dichlofluanid were considered to be the most appropriate for regulation. This approach was suggested and endorsed by the a*d-hoc* Environmental Panel, for the primary reason that post-approval control of amounts used is not possible and therefore a worst-case assumption is necessary. In addition to this, the only usage data currently available are those regarding the number of pleasure-craft located in U.K. waters; therefore, the inputs from ships would be additional. Finally, no allowance has been be made regarding the potential for additivity, synergism or antagonism between active ingredients which will coexist in the aquatic environment as a result of leaching from AFPs. This is considered to be additional support for the 100 % 'worst-case' usage approach.

Table 7.3 : Summary Of Dichlofluanid PEC:PNEC Ratios Based On 100 % Usage On Pleasure-craft

estuary PEC:PNEC		open marina PEC:PNEC	
mean (±STD)	% exceedence*	mean (±STD)	% exceedence*
0.05 (±0.1)	0	0.7 (±0.4)	14.3

* % Exceedence is the percentage of single samples which were shown to exceed the MEC:PNEC quotient of 1, demonstrating that the measured concentration of booster biocides was greater than the 'no effect concentration' for marine periphyton communities, and therefore indicating significant risk of adverse effects.

The above summary PEC data in Table 7.3 suggest that dichlofluanid would be of low concern with only 14.3 % of open marina sites reaching unacceptable levels.

7.3 SIGNIFICANCE OF RESULTS

7.3.1 WATER CONCENTRATIONS

The predicted data provided by the REMA model have allowed development of the risk assessment to take on board the maximum risk posed from the pleasure-craft use of dichlofluanid AFPs in the U.K.. Unfortunately, it has not been possible to predict the environmental concentrations resulting from the use of dichlofluanid on larger commercial vessels and ships. Therefore, the predicted concentrations at the 100 % use level (for pleasure -craft) are more likely to underestimate the environmental exposure resulting from AFP use.

Whilst some exceedences were predicted, the REMA model data have indicated that for dichlofluanid, use in AFPs may result in unacceptable environmental exposure in open marinas. However, refinement of the risk assessment by reducing the safety factor from 50 to 10 would make the assessment acceptable (providing no new toxicity studies significantly reduced the available NOEC). Therefore, chronic fish data in addition to outstanding data clarifications on metabolite data have been requested.

7.3.2 SEDIMENT CONCENTRATIONS

The $PEC_{sediment}$ data for dichlofluanid, derived by the REMA model, demonstrated that the sediment compartment was of low concern. Therefore, no additional data requirements have been requested to address fate, behaviour or toxicity in sediment.

7.3.3 SECONDARY AND SUB-LETHAL ECOTOXICOLOGY DATA

Potential secondary effects as a result of fish bioconcentration of dichlofluanid are not of concern considering the low BCFs and rapid depuration. The potential for indirect or sub-lethal effects (i.e. endocrine disruption) should be considered in all cases where prolonged exposure to concentrations below those shown to elicit known toxic effects are likely. This consideration should remain until reliable data can be obtained which demonstrates that such effects do not occur. This is especially important when considering the use of booster biocides which result in direct environmental exposure. Other sub-lethal effects such as respiratory distress have been reported. However whilst the *ad-hoc* Environmental Panel recognised that the significance of these data are difficult to determine, since assessments for endocrine disruption (and other sub-lethal effects) are as yet not available, they recommended that the potential for both these effects should not be ignored. Indeed, when the techniques become available the Panel Members agreed that persistent active ingredients used in AFPs (or their metabolites) should be reconsidered.

7.3.4 METABOLITE FATE AND EFFECTS DATA

Degradation of a parent compound is often where the considerations for environmental risk end. However, risk assessments should be conducted for all reported persistent major (>10%) metabolites. Without consideration of the metabolites, a full assessment of the risk to the marine environment from the use of AFPs cannot be considered complete. Therefore, data are required for dimethylaminosulfanilide in order to fully establish the risk of this major metabolite to the environment.

7.3.5 MIXTURES OF SUBSTANCES

AFPs usually contain a mixture of booster biocides with copper or TBT compounds. Therefore, the environment will be routinely exposed to such mixtures as a result of *in situ* leaching. Under the current regulations [COPR, 1986], AFPs are registered and approved on an active ingredient basis, and mixtures of active ingredients are not considered. General understanding of the possible effects arising from mixtures such as synergism, additivity and antagonism, is as yet not sufficiently developed. Also, when considering the number of AFPs and potential combinations of these in the field at any one time, the applicability of laboratory studies in gaining an understanding of effects in the field becomes so complex that a realistic assessment is unlikely. In addition to this, the chemistry of booster biocides in paint matrices is very complicated and caution would have to be applied in the interpretation of results.

A simpler approach may be to consider the mode of action of the active ingredients present in AFPs. However in the absence of data, mixtures will not be considered further within this risk assessment with the assumption that assuming 100 % use of single active ingredients takes at the very least additivity into account.

7.4 DATA REQUIREMENTS

The data requirements are based on data gaps or have been requested as a result of unacceptable high predictions of environmental exposure using the REMA model.

1. A study to address the chronic exposure of dichlofluanid to a suitable m arine/estuarine fish species.

2. Confirmation that dichlofluanid significantly degrades in marine sediment-water systems, and identification of any subsequent persistent metabolites.

3. Once the significance and persistence of the metabolites have been established, additional ecotoxicology, fate and behaviour data may be required in order to permit a risk assessment to the same level as that of the parent compound.

4. A study to address bioaccumulation of dichlofluanid in shellfish, in order to address the potential risks to consumers via the food chain.

5. Clarification that the leaching rate of the parent active substance used for the risk assessment is representative of levels in currently approved antifouling products.

8. EFFICACY

8.1 BACKGROUND AND INTRODUCTION

Two processes describing the fouling of an immersed surface by organisms are recognised in the literature, and are presented in 2 papers.

The first of these papers describes a classical view of fouling, in which fouling is reported to occur as a successional process. A freshly immersed surface rapidly adsorbs various organic molecules to form a conditioning film. This film facilitates subsequent colonisation by such micro-organisms as bacteria and diatoms, which results in the formation of a slime layer. The microfouling slime layer is subsequently succeeded by macrofouling. This can be of two types; 'hard' and 'soft' fouling. Common soft-fouling organisms include algae, sponges, tunicates and hydroids. Common hard-fouling organisms include barnacles, mussels, clams and tubeworms. Larvae of fouling organisms are more diverse and numerous in coastal areas than in the open sea, therefore the fouling challenge is more intense in these areas. [US Naval Institute, 1981]

An alternative theory to succession has been offered by other workers. This is a much more complex process depending on the relative amount and type of fouling organism present. The different organisms are in dynamic equilibrium with the immersed surface, and in its absence, with flocculation of 'marine snow' (waste material, dead micro-organisms etc. falling through the water column). There are a number of secondary driving forces and behavioural interactions in this model. For example, certain types of microbial fouling may actually inhibit the settlement of macrofouling organisms. [Clare *et al*., 1992]

Animal and weed fouling of vessel surfaces increases the drag on the hull. This results in increased fuel costs and a reduction in the vessel's speed and manoeuvrability. Beyond a certain level, fouling will require removal from the vessel by its owner during dry-docking. The performance of antifouling products may be judged as the maximum specified time that a vessel can spend 'in service' before having to be dry-docked, cleaned and recoated. For insurance purposes, commercial vessels have to be dry-docked at least every 5 years for inspection and maintenance. Therefore, the ideal antifouling product would protect a vessel for this length of time so that extra expense is not incurred as a result of more frequent dry-docking. The period of time between repainting can however vary quite considerably depending upon the chosen antifouling product formulation, and on the environment in which it is used. Various environments will present different fouling challenges.

Parameters which will influence the service time of an antifouling product include :

trading patterns (coastal or deep sea, turn around time, speed of vessel etc.);
fouling conditions (warm or temperate water);
physico-chemical conditions of the seawater (e.g. pH and temperature); and
coating type and film thickness.

8.2 ACTIVE SUBSTANCES / COATING TYPES USED IN ANTIFOULING PRODUCTS

Antifouling products can be broadly divided into two types; those that contain tributyltin (TBT) and those that are TBT-free.

TBT-free products typically contain copper or a copper compound such as copper (I) oxide (Cu_2O), or copper (I) thiocyanate (CuSCN) as the principal biocide. Since some of the common algae such as *Enteromorpha* species and *Amphora* species are tolerant of copper, this active ingredient is 'boosted' or enhanced by the presence of one or more organic biocides such as Irgarol 1051 or dichlorophenyl dimethylurea (diuron). These are usually algicides, but may in addition possess a wider spectrum of antifouling activity.

Copper is also used to enhance the performance of tributyltin (TBT) products because organisms such as *Ectocarpus* species and *Achanthes* species are tolerant of tributyltin oxide (TBTO). [Callow, 1990; Hunter, 1994]

The antifouling products can be further categorised into the following broad coating types :

conventional (soluble matrix);
contact leaching (insoluble matrix); and
ablative (TBT and TBT-free).

The categorisation of coating types outlined above is very generalised. It should be noted that apart from the TBT ablative (self polishing co-polymer or SPC) products, the majority of other antifouling products do not necessarily rely on one single coating technology. Instead, composites of different technologies have been developed by antifouling formulators to suit customer specifications and environmental requirements. Further detail and descriptors for the individual coating types can be found in Appendix 4.

The Antifouling Manufacturers Working Group of the European Confederation of Paint, Printers', Inks and Artists Colours Manufacturers Association (CEPE) have agreed maximum protection periods that can be expected for each antifouling coating type. The types of coating and maximum protection periods with respect to recommended dry docking intervals are summarised in Table 8.1.

Table 8.1. : Maximum Periods Of Service For Various Types Of Antifouling Product

type of formulation	conventional	contact leaching	TBT free ablative	TBT ablative (SPC)
maximum period of service	18 months	24 months	3 years	5 years

It should be noted that the maximum protection periods presented in Table 8.1 are a generalisation of maximum protection periods that may be achieved within these very broad groupings. In reality, these agreed intervals reflect a compromise position reached between CEPE members. In addition, Table 8.1 does not provide an indication of the level of performance that can be obtained by a product specified within those time periods, or the level of performance required by a particular specification. Performance ratings are heavily

dependent upon the particular coating being applied to specification (surface preparation, primers, undercoatings, dry film thickness etc.), trading and sailing pattern of the vessel and a wide variety of environmental factors.

8.3 APPROVED ANTIFOULING PRODUCTS CONTAINING DICHLOFLUANID

The compound dichlofluanid is an active ingredient which is added to antifouling products, especially tin-free products, to control the growth of algal, weed and animal fouling on moving or static objects in water. It is usually added to enhance the antifouling properties of products which have copper compounds as the principal active ingredient.

There are currently (November 1999) 23 approved antifouling products registered with HSE which contain dichlofluanid. These 23 product approvals are held by 7 Approval Holders. The distribution of dichlofluanid antifouling products by active ingredient is shown in Table 8.2.

Table 8.2 : The Distribution Of Dichlofluanid Antifouling Products By Active Ingredient

active ingredient(s)	no.	active ingredient(s)	no.
dichlofluanid/Cu_2O	9	dichlofluanid/Cu_2O/diuron	1
dichlofluanid/CuSCN	5	dichlofluanid/Cu_2O/diuron/ Irgarol 1051	1
dichlofluanid/Cu_2O/Irgarol 1051	1	dichlofluanid/diuron/zinc pyrithione	1
dichlofluanid/CuSCN/Irgarol 1051	1	dichlofluanid	1
dichlofluanid/CuSCN/Irgarol 1051/ diuron	2	dichlofluanid/CuSCN/Irgarol 1051/ zinc pyrithione	1

Information on the coating type of each product has been received for 21 of the 23 products that contain dichlofluanid. Although the products may often be formulated as a composite of different coating technologies, Approval Holders have categorised their formulations according to the coating types that best describes them. In addition, ALL Approval Holders have provided information on maximum dry-docking intervals for their products. Coating types and dry-docking intervals are presented in Table 8.3.

Table 8.3. : Coating Types And Dry-Docking Intervals For Products Containing Dichlofluanid

coating type	number of products having maximum recommended dry-docking intervals						
	3 wks	4 mths	6 mths	8 mths	12 mths	24 mths	36 mths
ablative (TBT-free)	-	2	1	4	1	-	1
contact leaching	1	-	-	-	2	1	2
conventional	-	-	-	-	6	-	-
unspecified	-	-	-	-	2	-	-

Table 8.3 shows that 2 contact leaching coating type products exceed CEPE agreed maximum dry-docking periods (see Table 8.1). "Unspecified" refers to a product where information has not been provided by the Approval Holder.

Concentration ranges of biocides in dichlofluanid products are presented in Table 8.4.

Table 8.4. : Concentration Ranges Of Biocides In Dichlofluanid Products

coating type	biocides (and range as appropriate) % w/w						no. of products
	Cu_2O	CuSCN	dichlofluanid	Irgarol 1051	diuron	zinc pyrithione	
ablative (TBT-free)	0 - 34	0 - 21.6	2 - 9.42	0 - 4.2	0 - 6.5	0 - 2	9
contact leaching	0 - 41.2	0 - 19.06	1 - 9	-	-	-	6
conventional	33 - 35	18.5 - 26.6	1.35 - 2.6	-	-	-	6
unspecified	13.68 - 15.99	-	0.7 - 1.42	-	-	-	2

Table 8.4 shows that the level of dichlofluanid does not exceed 9.42 % w/w.

8.4 EFFICACY DATA SUBMITTED IN SUPPORT OF THE REVIEW OF DICHLOFLUANID IN ANTIFOULING PRODUCTS

No efficacy data from laboratory (*in-vitro*) toxicity screening tests have been submitted. Data from simulated field tests have been submitted by two current Approval Holders.

These data have been evaluated to illustrate that the use of dichlofluanid (in combination with either copper as principal biocides) will result in antifouling products that demonstrate effective antifouling capability.

8.4.1` SIMULATED USE TESTS

Due to the accessibility of test sites (most companies have access to raft testing facilities) and ease of operation, raft testing has been widely adopted for antifouling trials. As such they are important indicators of the antifouling capability of biocidal active substances.

They are static tests conducted usually with the test formulation coated on to submerged panels. These tests are used to demonstrate the effectiveness of an antifouling product relative to an uncoated (blank) substrate. The method is not applicable to evaluate complete coating systems (especially the more advanced SPC or ablative technologies) or the relative lifetime of coatings; i.e. it does not serve to demonstrate the actual performance in-service. Raft tests can however simulate the use of a product on a yacht which will spend most of its time stationary (at anchor/berthed). [Unpublished, 1996 and 1997]

Efficacy test data generated in this way have been provided by Approval Holders. Studies have been conducted on a range of products. These products are representative of current approved formulations that contain dichlofluanid, both in terms of levels of Irgarol present in combination with copper based derivatives, and the coating type. Both the levels of Irgarol present, in combination with copper based derivatives as the principal biocide, and the coating types have been encompassed.

The studies were, unless stated otherwise, generated in accordance with the CEPE Antifouling Working Group Method of the Generation of Efficacy Data [Unpublished, 1993 - see Appendix 5]. In this method, results are presented using scoring and assessment systems devised by the individual companies or test houses (i.e. not to a uniform industry standard).

8.4.1.1 Conventional Coatings

A raft test was conducted according to the test method described in Appendix 6, Section 6.3. The test site was Oslofjorden in Norway. The test panels measured 200 x 400 cm and were composed of plastic or metal. The test coating was a conventional system containing 1.35 % w/w dichlofluanid and 33.00 % w/w Cu_2O. The coating thickness was 100 µm dry film. A separate test panel treated with a formulation containing 7.80 % w/w TBTO, 18.00 % w/w tributyltin methacrylate (TBTM) and 43.5 % w/w Cu_2O served as a positive control. All panels were immersed in seawater on a raft for a period of 28 weeks.

The results of the test are presented in Figures 8.1 and 8.2.

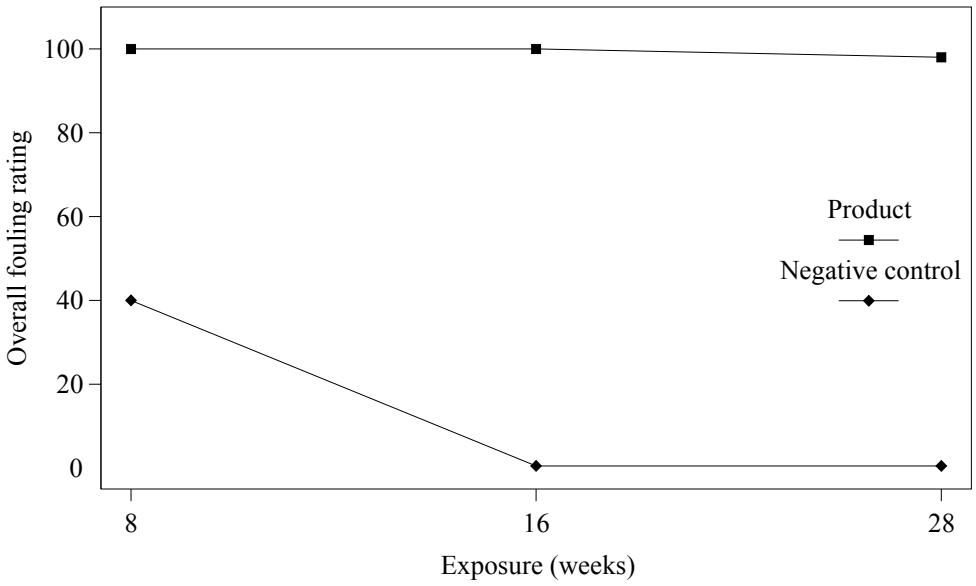


Figure 8.1 : Raft Test Of A Product Containing 1.35 % w/w Dichlofluanid And 33.0 % w/w Cu_2O

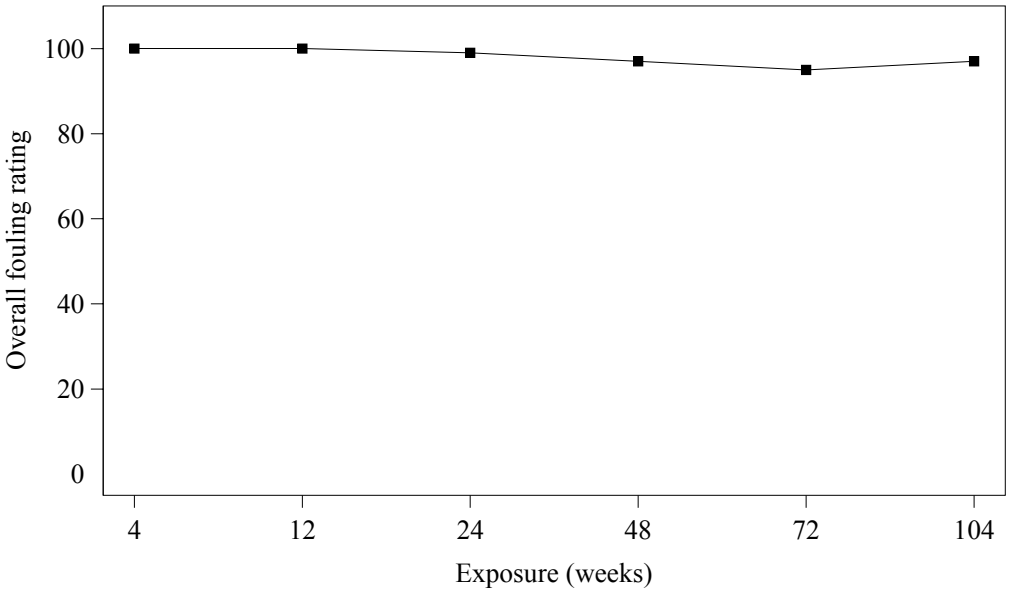


Figure 8.2 : Results Of Corresponding Positive Control Panel Treated With A Product Containing 7.80 % w/w TBTO, 18.00 % w/w TBTM And 43.50 % w/w Cu_2O

Figure 8.1 shows that following exposure of the test panel coated with the dichlofluanid containing product, the observed fouling gave rise to a calculated overall rating of 98-100 ("excellent") throughout the exposure period. This compared with a calculated overall rating of 0-40 ("poor") obtained for the negative (blank) control.

Figure 8.2 shows that following exposure of the test panel coated with the positive control formulation (containing TBTO, TBTM and Cu_2O); the observed fouling gave rise to a calculated overall rating of 95-100 ("very good" to "excellent") throughout the study. There are few data points in this study. However, the results provide some limited evidence of the efficacy of an antifouling containing dichlofluanid. [Unpublished, 1995(a)]

A second raft test was submitted by the company that had been conducted according to the test method described in Appendix 6, Section 6.3. Once again, the test site was Oslofjorden in Norway. The test panels measured 200 x 400 cm and were composed of plastic or metal. The test coating was a conventional system containing 1.35 % w/w dichlofluanid and 18.50 % w/w CuSCN. The coating thickness was 100 µm dry film. A separate test panel treated with a formulation containing 7.80 % w/w TBTO, 18.00 % w/w TBTM and 43.5 % w/w Cu_2O served as a positive control. All panels were immersed in seawater on a raft for a period of 28 weeks. The results of this test are presented in Figures 8.3 and 8.4.

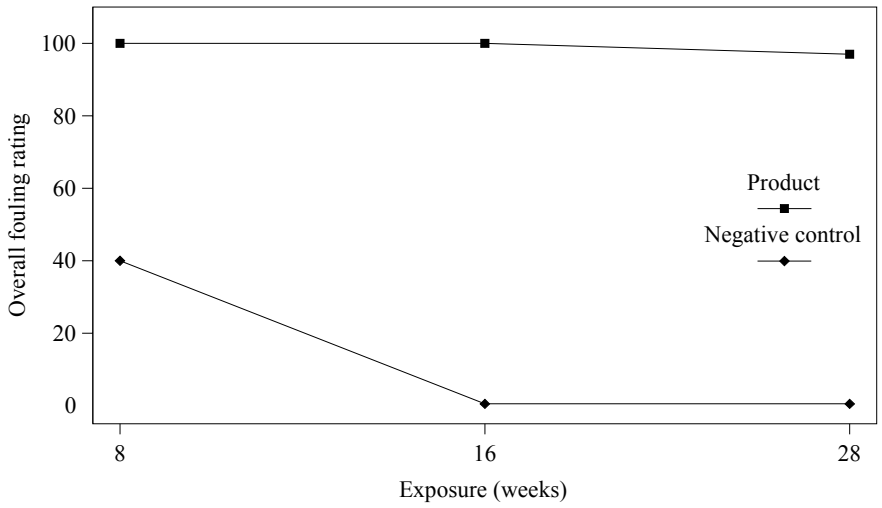


Figure 8.3 : Raft Test Of A Product Containing 1.35 % w/w Dichlofluanid And 18.50 % w/w CuSCN

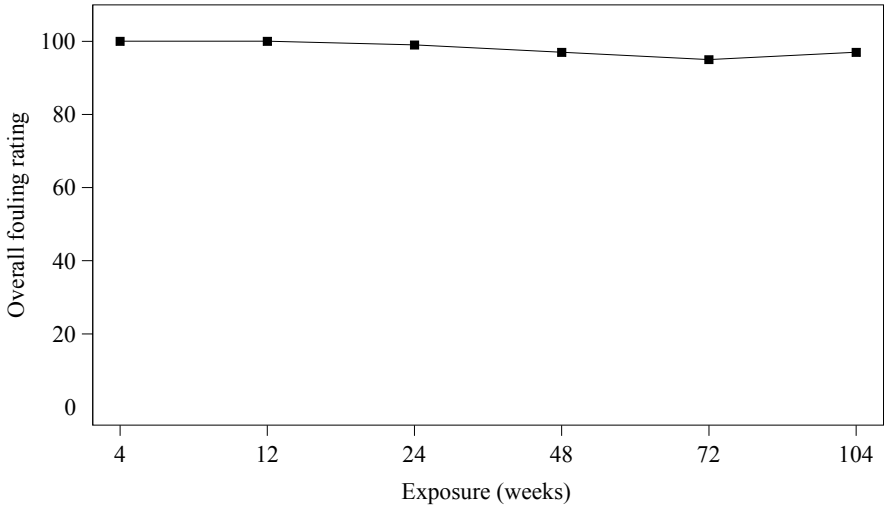


Figure 8.4 : Results Of Corresponding Positive Control Panel Treated With A Product Containing 7.80 % w/w TBTO, 18.00 % w/w TBTM And 43.5 % w/w Cu_2O

Figure 8.3 shows that following exposure of the test panel coated with the dichlofluanid containing product; the observed fouling gave rise to a calculated overall rating of 97-100 ("very good" to "excellent") throughout the exposure period. This compared with a calculated overall rating of 0-40 ("poor") obtained for the negative (blank) control.

Figure 8.4 shows that following exposure of the test panel coated with the positive control formulation (containing TBTO, TBTM and Cu_2O); the observed fouling gave rise to a calculated overall rating of 95-100 ("very good" to "excellent") throughout the exposure period.

Once again, there are few data points in this study. However, the results provide some limited evidence of the efficacy an antifouling containing dichlofluanid. [Unpublished, 1995b]

A third raft test was submitted by the company that had been conducted according to the test method described in Appendix 6, Section 6.3. Once again, the test site was Oslofjorden in Norway. The test panels measured 200 x 400 cm and were composed of plastic or metal. The test coating was a conventional system containing 2.60 % w/w dichlofluanid and 26.60 % w/w CuSCN. The coating thickness was 100 µm dry film. A separate test panel treated with a formulation containing 7.80 % w/w TBTO, 18.00 % w/w TBTM and 43.5 % w/w Cu_2O served as a positive control. All panels were immersed in seawater on a raft for a period of 28 weeks.

The results of this test are presented in Figures 8.5 and 8.6.

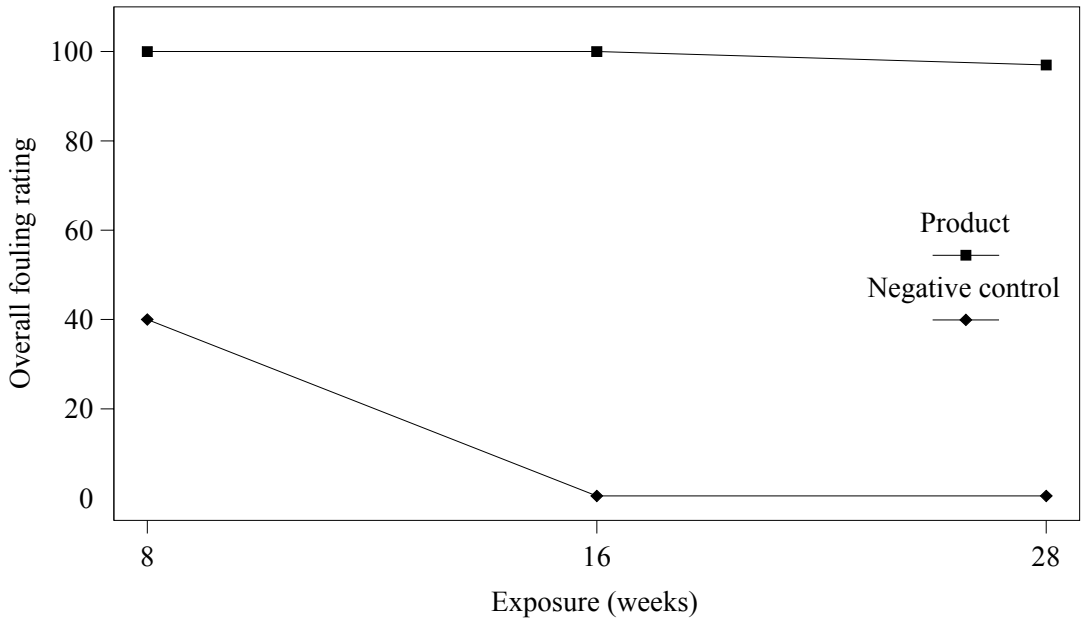


Figure 8.5 : Raft Test Of A Product Containing 2.60 % w/w Dichlofluanid And 26.60 % w/w CuSCN

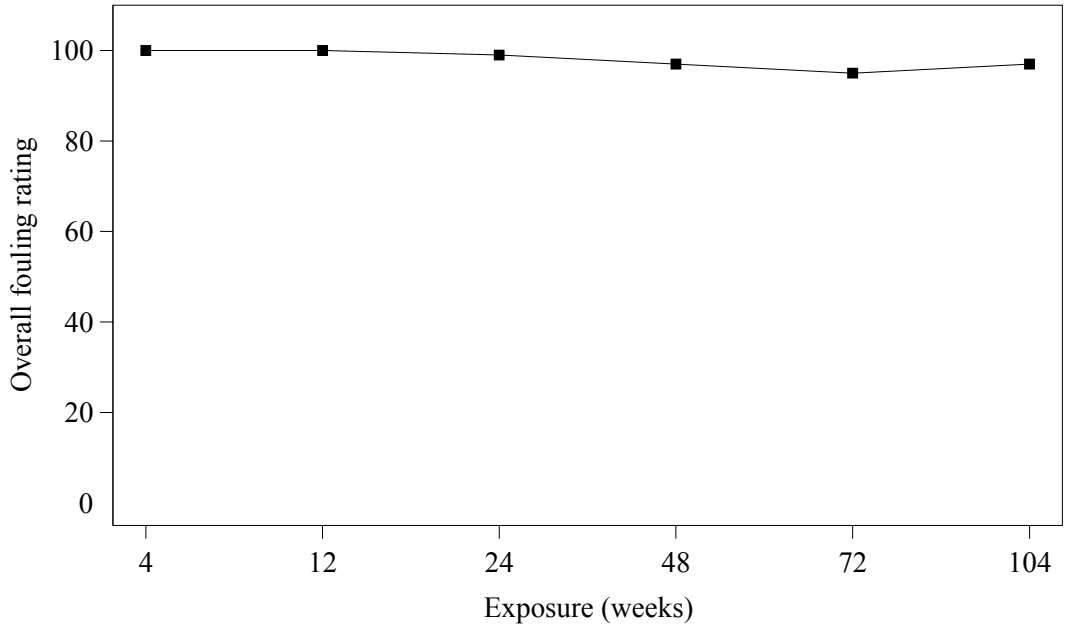


Figure 8.6 : Results Of Corresponding Positive Control Panel Treated With A Product Containing 7.80 % w/w TBTO, 18.00 % w/w TBTM And 43.5 % w/w Cu_2O

Figure 8.5 shows that following exposure of the test panel coated with the dichlofluanid containing product; the observed fouling gave rise to a calculated overall rating of 98-100 ("excellent") throughout the exposure period. This compared with a calculated overall rating of 0-40 ("poor") obtained for the negative (blank) control.

Figure 8.6 shows that following exposure of the test panel coated with the positive control formulation (containing TBTO, TBTM and Cu_2O); the observed fouling gave rise to a calculated overall rating of 95-100 ("very good" to "excellent") throughout the exposure period.

As with the previous 2 reports, there are few data points in this study. However, the results provide some limited evidence of the efficacy an antifouling containing dichlofluanid. [Unpublished, 1995c]

8.4.1.2 TBT-Free Ablative Coatings

A raft test was conducted according to the test method described in Appendix 8.3, Section 8.3.1. The test coating was a TBT-free ablative system containing 3.7 % w/w dichlofluanid and 39.0 % w/w Cu_2O. In addition to the negative control panel, a positive control treated with Cu_2O was also prepared. All panels were immersed in a vertical position, approximately

5 - 30 cm below the water line. The duration of the study was 104 weeks.

The results attributed to fouling of the test panels by slime, algae and animals are presented in Figure 8.7 for the test product, Figure 8.8 for the positive control and Figure 8.9 for the blank (negative control). Photographs of the individual test panels, taken at intervals throughout the study, were also submitted to HSE.

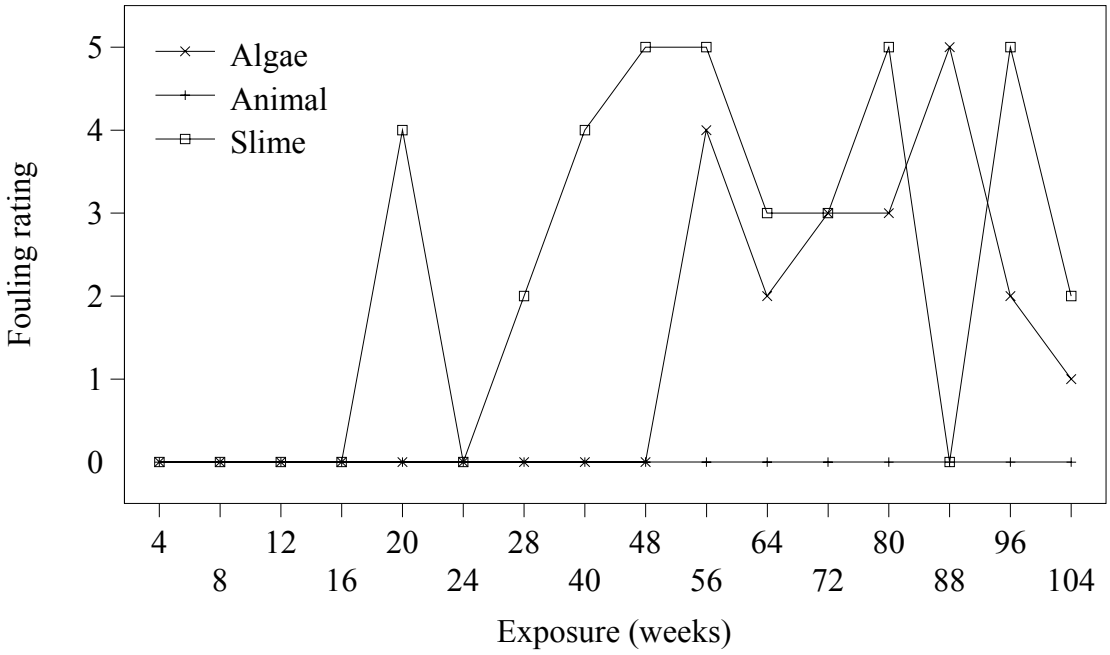


Figure 8.7 : Raft Test Of A Product Containing 3.7 % w/w Dichlofluanid And 39.0 % Cu_2O

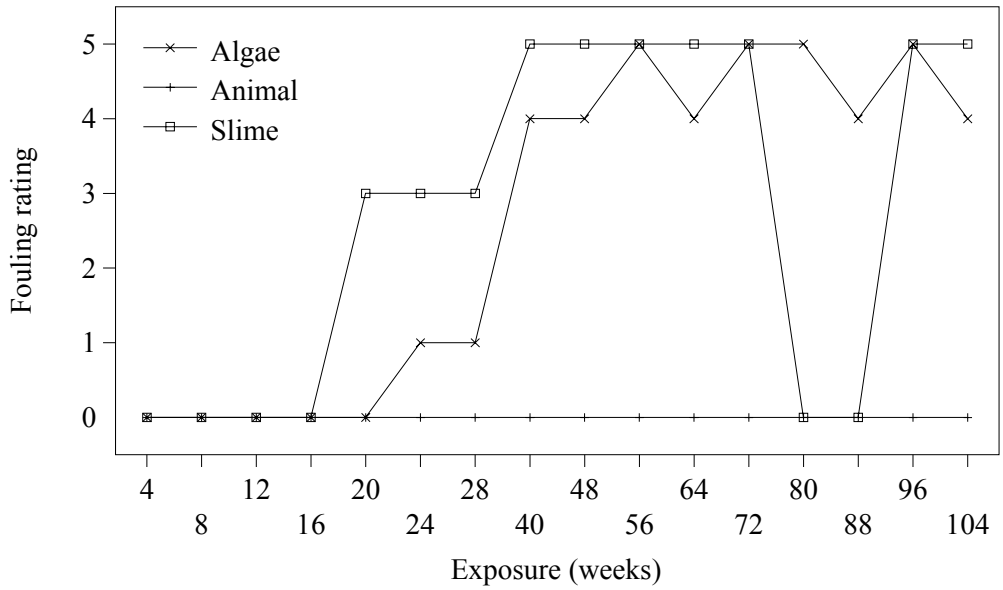


Table 8.8 : Raft Test Of A Product Containing 48.0 % Cu_2O (positive control)

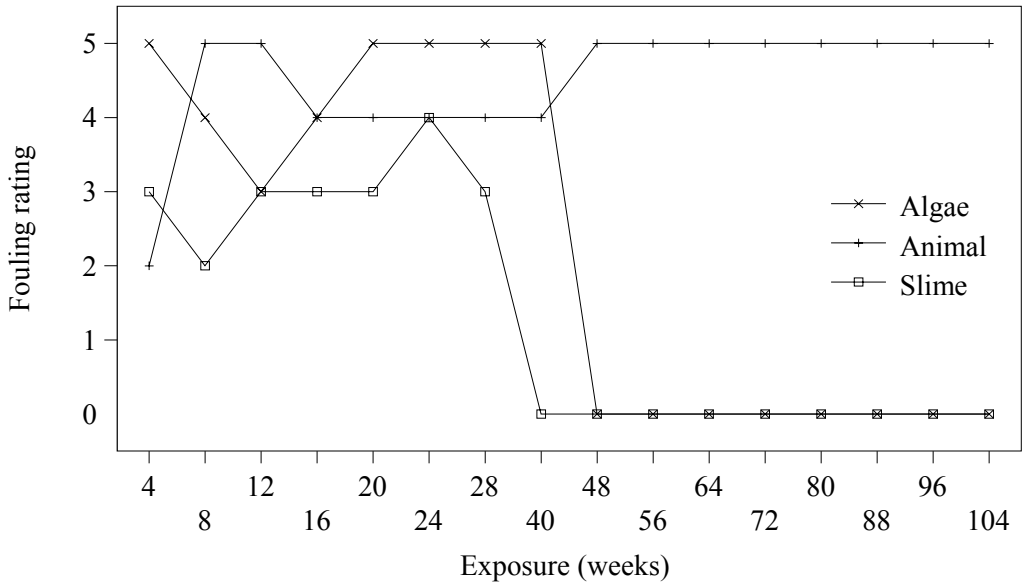


Figure 8.9 : Results Of Corresponding Blank Test Panel (negative control)

Figure 8.7 shows that following exposure of the test panel coated with the product, no fouling attributed to slime formation was recorded until week 20. Thereafter, although slime formation tended to fluctuate, this was generally high with a rating of 4 (maximum of 50 % fouling) recorded at weeks 20 and 40, and a rating of 5 (maximum of 100 % fouling) recorded at weeks 48, 56, 80 and 96. No fouling due to algae was recorded until week 56 when a rating of 4 was recorded, followed by a rating of 2 (maximum of 5 % fouling) in

weeks 64 and 96, a rating of 3 (maximum of 25 % fouling) in weeks 72 and 80, a rating of 5 in week 88, and a rating of 1 (maximum of 2 % fouling) in week 104. No animal fouling was recorded throughout the test period.

Figure 8.8 shows that following exposure of the test panel coated with a reference (positive control) product; no fouling attributed to slime formation was recorded until week 16. Thereafter, fouling was generally high with a rating of 3 recorded at weeks 20-28, a rating of 5 recorded at weeks 40-72 and 96-104, and a rating of 0 recorded at weeks 80-88. No fouling due to algae was recorded until week 24 when a rating of 1 was recorded. Thereafter, although a rating of 1 was recorded at week 28, fouling tended to be heavy, with a rating of either 4 or 5 recorded from week 40 until the end of the test period. No animal fouling was recorded throughout the test period.

Figure 8.9 shows that following exposure of the blank panel (negative control), a rating of 3 was recorded for slime formation at weeks 4, 12, 16, 20 and 28, a rating of 2 at week 8, and a rating of 4 at week 24. No slime formation was recorded from week 40 until the end of the test period. Fouling attributed to algae was generally high during the first half of the test period, with a rating of either 4 or 5 recorded until week 40, with the exception of week 12 when a rating of 3 was recorded. No algal fouling was recorded from week 48 until the end of the test period. Animal fouling tended to be heavy, with a rating of either 4 or 5 recorded from week 8 until the end of the test period.

Under the conditions of this study, the test formulation was very effective in preventing the development of animal fouling; no animal fouling was recorded on the test panel. Animal fouling on the blank (control) panel was considerable.

With fouling attributed to algae it was evident that the test formulation containing dichlofluanid prevented the onset of fouling for the first 48 weeks of exposure, as compared with the positive control formulation where fouling was first recorded at 24 weeks. Thereafter, although the degree of fouling recorded for the dichlofluanid containing formulation was generally high, this was less than that for the positive control formulation. The dichlofluanid containing formulation was seen to be more effective at preventing the formation of algal fouling than that of the blank (control) panel where heavy fouling was recorded during the first half of the exposure period. Finally, the results obtained for the treated panels indicated a greater degree of slime formation than that for either animal or algal fouling.

These data therefore indicate that, under the conditions of the test, dichlofluanid in the antifouling product demonstrated antifouling properties with a spectrum of activity against both algae and animals [Unpublished, 1995d].

8.5 SUMMARY OF EFFICACY DATA

Limited efficacy test data generated using raft tests have been provided. These raft tests were performed at test sites at two different geographical locations. The fouling challenge is likely to have been different at the two sites; although, details have not been provided for one of them and only limited information is available on the other.

Data were provided by ONLY ONE of the companies who are Approval Holders for products containing dichlofluanid. These data were generated from studies conducted on a limited range of products representative of current approvals in respect of the levels of the "booster" dichlofluanid (present in combination with copper derivatives as the principal biocide).

All but one of the studies involved the testing of conventional products, the exception being a study conducted using a TBT-free ablative coating. All of the formulations tested contained copper as the principal active ingredient (as Cu_2O or CuSCN) and dichlofluanid. No other combinations of active ingredients were represented.

The results from the single raft test conducted on the TBT-free ablative coating product demonstrated that a satisfactory level of antifouling performance could be achieved.

There were few data points in the studies conducted on conventional products. However, the results provide some limited evidence of the efficacy of an antifouling formulation containing dichlofluanid.

No data have been specifically provided in support of products formulated using contact leaching technologies.

8.6 DATA REQUIREMENTS

To support the continued use of antifouling products containing dichlofluanid, raft test or, alternatively, field trial/in service monitoring data are required. As appropriate these data should cover :

(i) The efficacy of TBT-free ablative, conventional and contact leaching antifouling formulations.

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9. OVERALL RECOMMENDATIONS AND DATA REQUIREMENTS

1. Subject to the fulfillment of data requirements, provisional approval be allowed to continue for the professional use of antifouling products containing dichlofluanid at a maximum formulation concentration of 10 % w/w by brush, roller, spray, spreader, and aerosol (containing up to 1.5% w/w dichlofluanid).

2. All professional operators exposed to antifouling products containing dichlofluanid should wear a disposable coverall with hood (providing head protection) and a second overall beneath this coverall of a contrasting colour to the antifouling product being applied. All bare skin should be covered. The disposable coverall should normally be used for no more than one spraying session. The second overall should be changed regularly and whenever product breakthrough has been detected.

3. Professional operators working with dichlofluanid-containing antifouling products should wear impermeable gloves of a type recommended by the antifouling manufacturer as suitable for use with the formulation. These gloves should be changed regularly, e.g. after one or two days' use. Operators should wear impermeable (non-slip) footwear that protects the lower leg.

4. Professional operators (sprayers) exposed to antifouling products containing dichlofluanid must wear RPE. Appropriate RPE includes air-fed respiratory equipment with combined protective helmet and visor to protect the skin of the head and neck. Impairment of vision should be avoided. For non-sprayers, the need for RPE should be informed by a COSHH assessment.

5. The following approval conditions are to appear on professional-use products' Notices of Approval and Schedules and they should be reflected on product labels using the following precautionary phrases :

WEAR SUITABLE PROTECTIVE CLOTHING (COVERALLS OF A CONTRASTING COLOUR TO THE PRODUCT BEING APPLIED, BENEATH A DISPOSABLE COVERALL WITH HOOD), SUITABLE GLOVES, AND IMPERVIOUS FOOTWEAR THAT PROTECTS THE LOWER LEG.

DISPOSE OF PROTECTIVE GLOVES after use.

If the product is to be applied by spray :

WEAR SUITABLE RESPIRATORY EQUIPMENT (such as air-fed respiratory equipment with combined protective helmet and visor) when spraying.

DO NOT BREATHE SPRAY MIST.

6. Due to concerns over the sensitising potential of dichlofluanid amateur use by spray and aerosol should be revoked.

7. Amateur use by brush, roller and spreader may continue provided that gloves are worn as a precautionary measure.

Continued approval should be subject to the following data requirements :

(i) **M**easurements and full test reports for technical dichlofluanid on boiling point; relative density; and surface tension.

(ii) Two-year storage stability studies at ambient temperature on representative formulations.

(iii) Analytical method to determine dichlofluanid in water with a limit of quantitation of 0.1 $\mu g\ l^{-1}$.

(iv) A study of the dermal penetration of dichlofluanid from formulations representative of approved antifouling products. This study may be carried out *in vivo* or *in vitro*.

(v) An overview of nephrotoxicity across species , taking into account any data on histopathology and the onset of effects, to determine whether a NOAEL more appropriate to the pattern of user exposure than that established in the 1-year dog study can be identified. If a more appropriate NOAEL cannot be identified from existing data, an oral study of shorter duration in the dog may be required.

(vi) A revised explanation of the mechanism for the formation of thyroid tumours and their relevance to humans.

vii) A study to address the chronic exposure of dichlofluanid to a suitable marine/estuarine fish species.

(viii) Confirmation that dichlofluanid significantly degrades in marine sediment-water systems, and identification of any subsequent persistent metabolites .

(ix) Once the significance and persistence of the metabolites have been established, additional ecotoxicology, fate and behaviour data may be required in order to permit a risk assessment to the same level as that of the parent compound.

(x) A study to address bioaccumulation of dichlofluanid in shellfish, in order to address the potential risks to consumers via the food chain.

(xi) Clarification that the leaching rate of the parent active substance used for the risk assessment is representative of levels in clurrently approved antifouling products.

(xii) Raft test or, alternatively, field trial/in service monitoring efficacy data. As appropriate these data should cover the efficacy of TBT-free ablative, conventional and contact leaching antifouling formulations.

Data have now been submitted in response to these data requirements (November 2002).

10. REFERENCES

Brennecke R. and Vogeler K. [1984]. "Method For Gas Chromatographic Determination Of Residues Of Different Fungicides In Water". Pflanzenschutz-Nachrichten Bayer, **37**, 46-67.

Unpublished, [1985]. "Water Solubility Of Dichlofluanid at 20 ^{o}C".

Unpublished, [1986(a)]. "Spectra Of The Active Ingredient".

Unpublished, [1986(b)]. "Assay Of 1,1-dichloro-N-[(dimethylamino)sulfonyl] -1-fluoro-N-phenylmethanesulfenamide".

Unpublished, [1986(c)]. "Thermal Stability Of The Agrochemical Active Ingredient Dichlofluanid".

Unpublished, [1987]. "Partition Coefficient Of Dichlofluanid (Euparen) At 21 ^{o}C".

Unpublished, [1988]. . "Vapor Pressure Dichlofluanid (KUE 13032 C) (Euparen)".

Unpublished, [1990]. "Analytical Methods".

Unpublished, [1993]. "Assay And By-products -HPLC Method, External Standard".

SECTION 3 : MAMMALIAN TOXICOKINETICS AND TOXICOLOGY

Borzelleca J. F., Capen C. C. and Hallagen J. B. (1978). Lifetime toxicity carcinogenicity study of FC &C red (erythrosine) in rats. Food and Chem Toxicol. 25, 723-733.

Doerge D. R. and Takazawa R. S. (1990). Mechanism of thyroid peroxidase inhibition by ehtylenethiourea. Chem Res Toxicol. 3, 98-101.

Hill N. R., Erdreich S. L., Orville E. P., Roberts A. P., Rosenthal L. S. and Wilkinson F. C. (1989). Thyroid folicular carcinogenesis. Fund and Appl Toxicol. 12, 629-697.

Jennings A. S., Schwartz S. L., Balter N. J., Gardner D. and Witorsch R. J. (1990). Effects of oral erythrosine (2',4',5',7'-tetraiodofluorescein) on the pituitary-thyroid axis in rats. Toxicol. Appl. Pharmacol. 103, 549-556.

Takayama S., Aihara K., Onodera T. and Akimoto T. (1986). Antithyroid effects of propylthiouracil and sulfamonomethoxine in rats and monkeys. Toxicol and App Pharmacol. 82, 191-199.

Unpublished, [undated]. An Assessment Of Fixed Material From A Two Year Dietary Study In Rats Treated With KUE 13032C.
Unpublished, [1964]. Report Of 4-Months Feeding Tests On Rats With Active Ingredient Bayer 47531.

Unpublished, [1966]. : Subchronic Toxicological Study On Dogs.

Unpublished, [1968]. Chronic Toxicological Studies On Rats.

Unpublished, [1969(a)]. 3-Generation Study On Rats.

Unpublished, [1969(b)]. Chronic Toxicity Studies On Dogs (Two-year Feeding Experiment).

Unpublished, [1974]. KUE 13032C : Studies For Embryotoxic And Teratogenic Effects On Rats Following Oral Administration.

Unpublished, [1977]. [^{14}C] Dichlofluanid (Active Ingredient Of Euparen) Biokinetic Study On Rats.

Unpublished, [1978(a)]. Shirasu Y., Moriya M. and Watanabe T. Report Of The Mutagenicity Study Of Dichlofluanid (Euparen)

Unpublished, [1978(b)]. Ecker W. Biotransformation Of [^{14}C] Dichlofluanid In The Rat.

Unpublished, [1978(c)]. Flucke W. Euparen (KU 13032c) : Studies For Acute Toxicity To Rats, Mice, Guinea Pigs, Rabbits And Cats.

Unpublished, [1978(d)]. KUE 13032C : Micronucleus Test On The Mouse To Evaluate For Mutagenic Effect.

Unpublished, [1979(a)]. . KUE 13032C : Salmonella/Microsome Test To Evaluate For Point Mutagen.

Unpublished, [1979(b)]. KUE 13032c : Cytogenic Study Of The Spermatogonia Of The Chinese Hamster To Evaluate For Mutagenic Effect.

Unpublished, [1980(a)]. Study On Guinea Pigs For Sensitising Effect ('Draize Test').

Unpublished, [1980(b)]. Study For Sensitising Effect (Magnusson and Kligman's Maximisation Test).

Unpublished, [1980(c)]. KUE 13032c : Sister Chromatid Exchange On The Chinese Hamster In Vivo To Evaluate For Mutagenic Effect.

Unpublished, [1980(d)]. : Study For Sensitising Effect In The Open Epicutaneous Test.

Unpublished, [1981(a)]. Effect Of Subchronic KUE 13032c (Euparen Active Ingredient) Administration On The Thyroid Function In Male Rats.

Unpublished, [1981(b)]. KUE 13032c : Subchronic Toxicological Study To Ascertain The Dose-Time-Effect Relationship In The effect On The Thyroid (Feeding Study Over 9 Weeks).

Unpublished, [1982(a)]. KUE 13032c : Chronic Toxicological Study In Mice (Feeding Experiment Over Two Years).

Unpublished, [1982(b)]. Statement to pkt IV/1.2.2 Of The BBA Application Form 'Details Of Effects On Man, Internal Company Experience'.

Unpublished, [1982(c)]. KUE 13032 c (Dichlofluanid) : Study For Primary Skin And Mucosa Irritant Effect.

Unpublished, [1982(d)]. Embryotoxicity Study In Rabbits With Oral Application Of KUE 13032 c.

Unpublished, [1984]. KUE 13032 c / Dichlofluanid : Salmonella/Microsome Test To Evaluate For Point Mutation.

Unpublished, [1985(a)]. Mutagenicity Evaluation Of KUE 13032 C (VM) In The Mouse Lymphoma Forward Mutation Assay [Final Report].

Unpublished, [1985(b)]. [Phenyl-UL-^{14}C] Dichlofluanid Biokinetics Part Of General Metabolism Study In Rats.

Unpublished, [1986(a)]. Acute Dermal Toxicity In Albino Rabbits.

Unpublished, [1986(b)]. KUE 13032 c / Dichlofluanid : In Vitro Cytogenetic Study On Human Lymphocyte Cultures To Evaluate For Chromosome-Damaging Effects.

Unpublished, [1986(c)]. Acute Inhalation Toxicity In Rats.

Unpublished, [1986(d)]. KUE 13032 c/Dichlofluanid : Dominant Lethal Test On The Male Mouse To Evaluate For Mutagenic Effects.

Unpublished, [1986(e)]. Structural Clarification Of Metabolites Of [ring-U-^{14}C] Dichlofluanid In Rat Faeces.

Unpublished, [1987]. Euparen : Epicutaneous Testing Of Volunteers To Investigate Skin Allergy-Inducing Potential.

Unpublished, [1988(a)]. KUE 13032c : In Vivo Cytogenetic Study Of The Bone Marrow In Chinese Hamsters To Evaluate For A Chromosome-Damaging Effect.

Unpublished, [1988(b)]. KUE 13032c : Spot Test On Cross-Bred C57B1/6J x T stock Mouse Fetuses To Evaluate For Induced Somatic Changes In The Genes Of The Coat Pigment Cells.

Unpublished, [1988(c)]. Statement On Mutagenicity Of KUE 13032c.

Unpublished, [1988(d)]. KUE 13032 c / Dichlofluanid : Mutagenicity Study For The Detection Of Induced Forward Mutations In The V79-HGPRT Assay In Vitro.

Unpublished, [1988(e)]. KUE 13032c (Dichlofluanid) : Review Of Assessments Of Toxicological Data.

Unpublished, [1988(f)]. . Ex Vivo Hepatocyte UDS Study With KUE 13032c.

Unpublished, [1989(a)]. A Teratology Study With Dichlofluanid (EUPAREN VM90) In The Rat.

Unpublished, [1989(b)]. Pers. Comm.

Unpublished, [1989(c)]. Chromosome Aberration Assay In Bone Marrow cells Of The Chinese Hamster With KUE 13032c.

Unpublished, [1990]. KUE 13032c (Dichlofluanid) : Study For Acute Oral Toxicity In Rats.
Unpublished, [1991(a)]. Statement On The Publication Of Heil et al., 'Genotoxicity Of The Fungicide Dichlofluanid In Seven Assays', Environmental and Molecular Mutagenesis 17, 20-26.

Unpublished,[1991(b)]. KUE 13032c:Two-Generation Study On Rats.

Unpublished, [1992(a)]. Technical Grade Dichlofluanid: Oral Dosing Chronic Toxicity Studies In The Beagle Dog.

Unpublished, [1992(b)]. KUE 13032 c : Supplementary Two-Generation Study On Rats.

Unpublished, [1993(a)]. Toxicological assessment Of Dichlofluanid : Opinion On New Findings With Regard To Long Term Effects.

Unpublished, [1993(b)]. Study On Chronic Toxicity And Carcinogenicity In Wistar Rats (Administration In Food For 105 Weeks).

Unpublished, [1993(c)]. Preventol A 4-S : Particle Size Distribution.

Unpublished, [1993(d)]. Study For Oncogenicity In B6C3F1 Mice (Administration In Feed Over 2 Years).

Unpublished (1995) *In vitro* characterisation of the goitrogenic properties of its metabolite 2-thiazolidinethione-4-carboxylic acid (TTCA). .

SECTION 4 : OPERATOR AND 'CONSUMER EXPOSURE AND RISK ASSESSMENT

Boxall, A.B.A., A.U. Conard and S. Reed. [Undated]. Environmental Problems from Antifouling Agents, Survey of Manufacturers, Chandlers (Suppliers) and Treatment Sites. Published R&D Technical Report P215. Commissioned by the Environment Agency

Garrod ANI, Guiver R and Rimmer DA (2000). Potential exposure of amateurs (consumers) through painting wood preservative and antifoulant preparations. Ann Occ Hyg **44**(6), 421-426.

HSE (2000). Dermal exposure to non-agricultural pesticides. Exposure assessment document (EH 74/3). HSE Books, Sudbury, UK ISBN 0-7176-1718-1.

MAFF [1992] Predictive Operator Exposure Model (POEM) V 1.0. DEFRA Pesticides Safety Directorate, Mallard House, King's Pool, 3 Peasholme Green York. YO1 2PX

Unpublished, [1994]. Occupational hygiene assessment of exposure to insecticides and the effectiveness of protective clothing during sheep dipping operations.

Unpublished, 1995. 'Determination of exposure to tin during commercial application of antifouling paint to ship hulls.'

Unpublished, [1996]. Assessment of exposure to non-agricultural pesticides and effectiveness of protective clothing.

Unpublished, [1998]. Measurement of potential dermal exposure in Greece and Spain with patch and whole body dosimetry techniques.

SECTION 5 : ENVIRONMENTAL FATE AND BEHAVIOUR AND

Callow, M. E. and Finlay, J. A. [1995]. A simple method to evaluate the potential for degradation of antifouling biocides. Biofouling. Vol. 9 (**2**): 153-165.

Unpublished, [undated]. Overall result of studies to investigate fate of Euparen WP (Dichlofluanid) in soil (laboratory degradation studies

Unpublished, [1975]. Behaviour of pesticides in soil.

Unpublished, [1982]. Properties of pesticides in water.

Unpublished, [1985]. Leaching characteristics of Dichlofluanid (Euparen^{R}) aged in soil. .

Unpublished, [1987(a)]. Metabolism of [ring-UL-^{14}C] Dichlofluanid (Euparen^{R}) in soil under anaerobic conditions.

Unpublished, [1987(b)]. Leaching characteristics of Dichlofluanid (Euparen^{R}) with various modes of application.

Unpublished, [1988]. Metabolism of [benzene ring-UL-^{14}C] Dichlofluanid (Euparen^{R}) in soil under aerobic conditions.

Unpublished, [1991]. R. Grau. Dichlofluanid - Bioconcentration in fish.

SECTION 6 : ECOTOXICOLOGY

Antychowicz, J. Szymbor, E. and Roszkowski, J. [1979]). Investigations upon the effects of some pesticides on carp (*Cyprinus carpio*). Bull. vet. Inst. Pulawy. Vol. 2 (3-4): 124-130.

Unpublished, [1985]. Growth inhibition of green algae (*Scenedesmus subspicatus*) by Dichlofluanid (90 % premix).

Unpublished, [1986(a)]. Acute flow-through toxicity of dichlofluanid to *Daphnia magna*.

Unpublished, [1986(b)]. Acute flow-through toxicity of dichlofluanid to bluegill sunfish (*Lepomis macrochirus*).

Unpublished, [1986(c)]. Acute flow-through toxicity of dichlofluanid to rainbow trout (*Onchorhynchus mykiss*).

Unpublished, [1986(d)]. Acute oral LD_{50} of dichlofluanid to Bobwhite quail.

Unpublished, [1986(e)]. . Subacute dietary LC_{50} of dichlofluanid to Mallard ducks.

Unpublished, [1986(f)]. Subacute dietary LC_{50} of dichlofluanid to Bobwhite quail.

Unpublished, [1987]. Toxicity to bacteria.

Unpublished, [1989]. Influence of Dichlofluanid on the reproduction of *Daphnia magna*.

SECTION 7 : ENVIRONMENTAL RISK ASSESSMENT

ACP [2002]. Environmental Risk Assessment of Booster Biocides in Antifouling Products. Advisory Committee on Pesticides Published Evaluation Document No. 205, available from Department for Environment, Food and Rural Affairs, Pesticides Safety Directorate, Mallard House, Kings Pool, 3 Peasholme Green York YO1 7PX.

EURATGD, [1996]. Technical Guidance Document in Support of Commission Directive 93/97/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances. Part II. ECSC-EC-EAEC, Brussels. Luxembourg.

SECTION 8 : EFFICACY

Callow M. [1990]. “Ship Fouling : Problems And Solutions”. Chemistry & Industry, 5, pp 123 - 127, 5th March 1990.

Clare A.S., Rittschof D., Gerhart D.J. and Maki, J.S. [1992]. “Molecular Approaches To Non-Toxic Antifouling”. Invertebrate Reproduction and Development 22:1-3, 67-76.

Hunter J.E. [1994]. “Biocides Directive Steering Group - Antifouling Coatings”. Paper presented to CEPE Antifouling Manufacturers Working Group.

Unpublished, [1993]. “CEPE Antifouling Working Group Method For The Generation Of Efficacy Data”.

Unpublished, [1995(a)]. “Documentation Of Efficacy.

Unpublished, [1995(b)]. “Documentation Of Efficacy.

Unpublished, [1995(c)]. “Documentation Of Efficacy.

Unpublished, [1995(d)]. “Antifouling Coatings - Performance Report.

Unpublished, [1996 and 1997]. Pers. Comm.

US Naval Institute [1981]. “Marine Fouling And Its Prevention". Annapolis: Woods Hole Oceanographic Institute, US Naval Institute, 165-207.

11. APPENDICES

APPENDIX 1

CALCULATIONS FOR EXPOSURE ASSESSMENT

The following database models were initially presented in guidance document EH73/3 (HSE, 2000).

Defaults assumed - spray applications.

weight of operator	60 kg
operator respired volume	1.25 m^3 h^{-1}
average length of shift	3 h (professional)

Table A1.1 : Summary Of Exposure Data For Sprayers (mg h^{-1} in-use product)

	frequency	central tendency	95th% or * worst case
potential dermal	100 %	6170	**44700**
weighted indicative value		**6170**	
clothing penetration	93 %	4.5 %	
weighted indicative value		**4 %**	
in-glove exposure	100 %	60	**241**
weighted indicative value		**60**	
inhalation exposure	91 %	6.6 mg m^{-3}	**64.6 mg m^{-3}**
weighted indicative value		**6 mg m^{-3}**	

Table A1.2 : Summary Of Exposure Data For Pot-Men (mg h^{-1} in-use product)

	frequency	central tendency	95th% or * worst case
potential dermal	100 %	2940	**15000**
weighted indicative value		**2940**	
clothing penetration	59 %	7 %	
weighted indicative value		**4 %**	
in-glove exposure	100 %	34.9	**1380 ***
weighted indicative value		**35**	
inhalation exposure	68 %	0.9 mg m^{-3}	**42 mg m^{-3} ***
weighted indicative value		**0.6 mg m^{-3}**	

Table A1.3: Summary Of Exposure Data For Ancillary Workers (mg h^{-1} in-use product)

	frequency	central tendency	95th% or * worst case
potential dermal	100 %	885	**3470**
weighted indicative value		**885**	
clothing penetration	59 %	7 %	
weighted indicative value		**4 %**	
in-glove exposure	100 %	35	**180 ***
weighted indicative value		**35**	
inhalation exposure	50 %	1.7 mg m^{-3}	**4.8 mg m^{-3} ***
weighted indicative value		**0.8 mg m^{-3}**	

The original reports from which these tables' data are derived are available for scrutiny at HSE Bootle. The number of exposure data and the exposure ranges are shown in Table A4.4

Table A1.4 : Summary Of Exposure Data And Ranges - Antifoulant Spraying

exposure	sprayer		pot-men and other operators	
	no of data	range	no of data	range
potential dermal (mg h^{-1} product)	29	52.2 - 74100	28	16.2 - 18200
in-gloves (mg h^{-1} product)	19	0.18 - 252	17	0.31 - 1380
inhaled (mg m^{-3} product)	20	0.04 - 79.4	16	0.04 - 41.6

Table A1.5 : Exposure Estimate (Product) - Sprayers

	central tendency	worst case
Coveralls		
potential dermal exposure (mg h^{-1})	6170	44700
work time per day (h)	3	3
daily deposit on clothes	18510	134000
penetration (%)	4	4
dermal exposure to product (mg)	740	5360
Gloves		
dermal exposure inside (mg h^{-1})	60	241
work time per day	3	3
dermal exposure to product (mg)	180	723
Total dermal exposure		
antifoulant product (mg)	**920**	**6080**
Inhaled		
concentration (mg m^{-3})	6	64.6
work time per day (h)	3	3
inhaled air volume (m^3)	3.75	3.75
inhaled product, mg - no RPE	**22.5**	**242**
inhaled product, mg - RPE x PF 50	**0.45**	**4.84**

PF is RPE protection factor

Table A1.6 : Exposure Estimate (Product) - Pot-Men

	central tendency	worst case
Coveralls		
potential dermal exposure (mg h^{-1})	2940	15000
work time per day (h)	3	3
daily deposit on clothes	8820	45000
penetration (%)	4	4
dermal exposure to product (mg)	353	1800
Gloves		
dermal exposure inside (mg h^{-1})	35	1380
work time per day	3	3
dermal exposure to product (mg)	105	4140
Total dermal exposure		
antifoulant product (mg)	**458**	**5940**
Inhaled		
concentration (mg m^{-3})	0.6	42*
work time per day (h)	3	3
inhaled air volume (m^3)	3.75	3.75
inhaled product, mg - no RPE	**2.25**	**158***

***** these values reduce to 3.84 mg m^{-3} and 14.4 respectively when the top data is disregarded as proposed in the text

Table A1.7 : Exposure Estimate (Product) - Ancillary Workers

	central tendency	worst case
Coveralls		
potential dermal exposure (mg h^{-1})	885	3470
work time per day (h)	3	3
daily deposit on clothes	2660	10400
penetration (%)	4	4
dermal exposure to product (mg)	106	416
Gloves		
dermal exposure inside (mg h^{-1})	35	180
work time per day	3	3
dermal exposure to product (mg)	105	540
Total dermal exposure		
antifoulant product (mg)	**211**	**956**
Inhaled		
concentration (mg m^{-3})	0.8	4.8
work time per day (h)	3	3
inhaled air volume (m^3)	3.75	3.75
inhaled product, mg - no RPE	**3**	**18**

Brush And Roller Applications

defaults assumed :

user weight 60 kg
operator respired volume 1.25 m^3 h^{-1}
median job duration 1.5 h

professional chandlers undertake the same tasks as amateur users of antifoulants

Table A1.8 : Summary Of Exposure Data For Amateur Users (mg h^{-1} in-use product)

	frequency	central tendency	95th% or * worst case
potential dermal	100 %	1020	**6480 ***
weighted indicative value		**1020**	
clothing penetration	11 %	42 %	
weighted indicative value		**5 % ******	
in-glove exposure	100 %	31.2	**1110 ***
weighted indicative value		**31**	
bare hand exposure	100 %		**4400 ***
inhalation exposure	44 %	0.04 mg m^{-3}	**0.11 mg m^{-3} ***
weighted indicative value		**0.02 mg m^{-3}**	

** It is possible that amateurs wear only minimal clothing when applying antifoulant products. A weighted indicative value for penetration in such cases is a default **50 %**.

Table A1.9 : Exposure Estimate (Product) - Amateur Users

	central tendency	worst case
Coveralls		
potential dermal exposure (mg h^{-1})	1020	6480
work time per day (h)	1.5	1.5
daily deposit on clothes	1530	9720
penetration (%)	5	50 **
dermal exposure to product (mg)	76.5	4860
Gloves		
dermal exposure inside (mg h^{-1})	31	4400 **
work time per day	1.5	1.5
dermal exposure to product (mg)	46.5	6660
Total dermal exposure		
antifoulant product (mg)	**123**	**11500**
Inhaled		
concentration (mg m^{-3})	0.02	0.11
work time per day (h)	1.5	1.5
inhaled air volume (m^3)	1.88	1.88
inhaled product, mg - no RPE	**0.04**	**0.21**

** worst case - no gloves, minimal clothing.

Table A1.10 : Exposure Estimate (Product) - Professional Chandler

	central tendency	worst case
Coveralls		
potential dermal exposure (mg h^{-1})	1020	6480
work time per day (h)	1.5	1.5
daily deposit on clothes	1530	9720
penetration (%)	5	5
dermal exposure to product (mg)	76.5	486
Gloves		
dermal exposure inside (mg h^{-1})	31	1110
work time per day	1.5	1.5
dermal exposure to product (mg)	46.5	1670
Total dermal exposure		
antifoulant product (mg)	**123**	**2160**
Inhaled		
concentration (mg m^{-3})	0.02	0.11
work time per day (h)	1.5	1.5
inhaled air volume (m^3)	1.88	1.88
inhaled product, mg - no RPE	**0.04**	**0.21**

APPENDIX 2

TOXICITY OF DICHLOFLUANID TO FRESHWATER SPECIES

species	test material	nominal (N) / mean (M) concs.	GLP	end-point mg l^{-1}	reference
Scenedesmus subspicatus (freshwater algae)	dichlofluanid (90 % premix)	N	✓	96 h E_bC_{50} >1 96 h E_rC_{50} >1 96 h NOEC 1.0	unpublished 1985b
Daphnia magna (water flea)	dichlofluanid Preventol A4S (88.5 %)	M	✓	24 h EC_{50} 0.57 48 h EC_{50} 0.42 48 h NOEC 0.07	unpublished 1986a
Daphnia magna (water flea)	dichlofluanid (91.4 %)	N		24 d NOEC 0.04 24 d LOEC 0.20	unpublished 1989
Lepomis machrochirus (bluegill sunfish)	dichlofluanid Preventol A4S (88.5 %)	M	✓	96 h LC_{50} 0.030 NOEC <0.024	unpublished 1986b
Oncorhynchus mykiss (rainbow trout)	dichlofluanid Preventol A4S (88.5 %)	M	✓	96 h LC_{50} 0.010 NOEC <0.0026	unpublished 1986c

APPENDIX 3

USE OF SAFETY FACTORS IN ENVIRONMENTAL RISK ASSESSMENT

By assuming that ecosystem sensitivity depends on the most sensitive species and that by protecting the community structure the function is also protected, single-species acute toxicity data are extrapolated to ecosystem effects. The most sensitive species is generally established in the laboratory.

Safety factors are applied to the generated toxicity data to predict a concentration below which the probability of environmental effects is considered sufficiently low to accept the proposed use of a product. Note : this is not a concentration below which an active ingredient is considered to be safe. In deciding the size of the safety factor to use with data several uncertainties must be addressed :

inter-species variations

acute to chronic toxicity extrapolation

laboratory to field data extrapolation

The following safety factors could have been, and were, used during the environmental risk assessment of the booster biocides :

v 1000* applied to the lowest L/EC_{50} of the core data set, i.e. acute toxicity of the active ingredient to fish, daphnids and algae. A safety factor of 1000 is conservative and protective, and is used when only the core set of acute data are available.

v 50* applied to the lower NOEC from long-term toxicity data for two species from two taxonomic groups.

v 10* applied to the lowest NOEC from long-term toxicity data for fish, daphnids and algae. With increasing availability of acute and chronic data the safety factor of 50 or even 10 can be considered.

v 100** applied to the lowest NOEC from long-term toxicity data for two or more species from two or more taxonomic groups when the majority of the assessed data are published rather than company supplied safety data.

v

v * these safety factors are as described in the EURATGD document.

v ** this safety factor has been previously used by HSE, because the quality of published data is considered to be less assured than that of safety data generated in a quality controlled laboratory. It is a precautionary approach that has been previously accepted by the IDS and ACP. [EURATGD, 1996]

APPENDIX 4

CURRENT ANTIFOULING COATING TYPES

coating type	description and properties
soluble matrix (conventional)	In coatings of this type the biocide(s) have been physically mixed ('freely associated') into a rosin matrix. Upon exposure to seawater, the slightly acidic matrix slowly dissolves releasing the biocide(s) into the water (seawater is slightly alkaline, pH 8, and the acidic matrix readily dissolves). Continuous dissolution of the coating surface occurs, resulting in fresh biocide(s) being released until eventually the film is exhausted. The soluble matrix coatings have poor mechanical properties which limit film thickness and hence, the coating lifetime attainable to approximately 12-18 months. As the matrix rosin is a natural product, batches differ and therefore coating lifetime is unpredictable.
insoluble matrix (contact leaching or long life)	Within this type of coating, the binder or matrix is insoluble, and the biocide(s) are physically mixed into the matrix (often at higher concentrations than is the case with the conventional coatings). As seawater enters the paint film, the biocides are released by dissolution and diffusion from within the insoluble matrix. This type of coating has a high initial release rate, which decreases exponentially with time as the biocide(s) have further to travel through the paint film. This release process continues until exhaustion of the coating. The higher mechanical strength obtained with these coatings allow applications of thicker systems and, as a consequence, coating lifetimes of approximately 24 months are attainable.
ablative (self polishing copolymer) TBT coatings	In this type of coating the TBT biocide is chemically bound to the binder of the paint; a methacrylic acid/methylmethacrylate copolymer matrix into which other biocides can be incorporated. The copolymer hydrolyses at a predictable rate in seawater (depending on temperature, pH and rate of movement of a vessel through water), releasing the biocide(s) into the surrounding water and creating a localised concentration at the paint surface discouraging the growth of settling organisms. This hydrolysis results in a softening of the surface layer of the copolymer and, together with the physical wearing away of the binder by the action of passing seawater (‘polishing’), exposes fresh surface layers. This mode of action with biocide release and polishing rate are both dependent on the same (chemical) process. The paint film thus smoothes, reducing drag and turbulence until eventually through these processes, the whole of the coating is exhausted. After initial rapid release, a steady biocide release is achieved; the life of the coating is proportional to its thickness and is accurately predictable.
coating type (continued)	**description and properties (continued)**
	The copolymer has a high mechanical strength allowing build up of very thick systems and hence, correspondingly long coating lifetimes; typically up to 5 years.

ablative (polishing copolymer) tin-free coatings	Coatings of this type rely on soluble medium, such as rosin, in combination with insoluble polymers to form a matrix which wears away *physically* at a controlled rate. The biocide(s) are mixed into the matrix and released by dissolution at a rate determined by the rate of physical ablation of the polymer. The physical ablation process is less controlled and predictable than the chemical ablation process. Therefore the steady release rate, predictable life, smoothing and recoating properties of the TBT copolymer coatings are difficult to achieve with this group of coatings. These tin-free copolymer coatings have to date demonstrated that dry docking intervals of 3 years can be achieved by the better performers within this group of products.

APPENDIX 5

ANTIFOULING COATINGS: METHOD FOR THE GENERATION OF EFFICACY DATA - [Unpublished, 1993]

Scope

The purpose of this test method is to determine, by raft testing, the effectiveness of an antifouling coating relative to an untreated substrate.

In static raft testing, the fouling challenge varies between raft sites, between positions on rafts, and from season to season etc.. The results obtained by the raft testing described in this method are purely an indication of a product's ability to prevent the settlement of fouling organisms under static conditions at a particular fouling challenge, relative to an uncoated substrate tested under exactly the same conditions.

The present method is not applicable to evaluate complete coating systems or the relative lifetime of coatings. Thus, the results obtained by the described method are not serving to demonstrate actual performance in service. The results obtained cannot therefore be adopted for the relative assessment of products.

Principle of Method

In this method, the antifouling paint is applied onto one or more raft test panels and exposed from the raft along with an uncoated substrate. Raft panels are made out of hard material; typically plastic or another inert material. Application of paint is made by brush, roller, spray, or other specialised equipment such as a bar-type applicator. Minimum dry film thickness should be in the range 90-100 μm, although thicker films may be applied depending on the decided use pattern of the antifouling coating. Minimum area for immersion is 150-200 cm^2. After proper drying, the panels in test are mounted on the raft and immersed in the marine environment. Depending on the intended use pattern of the antifouling paint, one or more raft sites may be used for exposure.

At given time intervals the panels are assessed for presence of fouling organisms. Assessment of slime, algae and animal fouling is quantitative or, as a minimum, semi-quantitative. The duration of the test may vary. To demonstrate efficacy the minimum immersion time for testing is 6 months. Depending on the intended use pattern of the paint under test, the immersion time may be extended.

Resistance to fouling at the raft site is demonstrated if there is no colonisation of the surface, or colonisation is minimal relative to the uncoated substrate.

Fouling assessment data are reported in the form of a raft performance report.

APPENDIX 6

METHODS FOR EFFICACY TESTING

6.1 RAFT TEST METHOD 1

The test product is applied by hand to a 200 cm^2 acrylic panel. Panels are coated on one side with one coat giving a dry film thickness of approximately 80 - 100 µm (the actual area of panel coated is approximately 132 cm^2). A second, identical panel is left blank as a negative control.

After the panels are painted they are left to dry for 1 week before sealing in a plastic bag for transport to the test facility on the Mediterranean for immediate immersion. The panels are hung vertically on a test raft floating on the surface of the sea, and submerged a minimum of 1.5 cm apart, facing in the same direction. The site is reported to represent medium to heavy fouling conditions. No further environmental details of the test site have been provided.

Evaluation of panel fouling is usually undertaken monthly, and photographs (submitted to HSE) are taken every second month. The panels are removed from the sea and sprayed with a hand held shower at a distance of 60 cm to remove silt and loosely attached organisms. A visual assessment is made for slime (composed of diatoms and initial algal germination), algal and animal fouling. The percentage of fouling for each of the above 3 groups of organisms is rated as indicated in Table 6.1.1.

Table 6.1.1 : Rating Of Fouling Assessment In Raft Tests Conducted By The Company

percentage of fouling	rating
no fouling	0
0-2	1
2-5	2
5-25	3
25-50	4
50-100	5

The sum of all the fouling rates for each group gives an estimation of the antifouling properties of the test product.

The performance of the products is contrasted against a negative (blank) control.

6.2 RAFT TEST METHOD 2

Two coats of the product are applied by brush to a marine plywood panel. Two separate panels are coated for immersion at each test site. A negative (blank) control panel serves as a control at each test site.

UK test site 1

Typical water temperatures, at UK test site 1, range from a winter low of about 9 ^{o}C in February/March to a summer high of about 19 ^{o}C in August. Salinity is generally in the range 32-34 % but may on occasion drop below 30 % immediately after a heavy rainfall. The sea water pH is generally in the range 8.10-8.25. The tidal range is 2 m at low tide to 7 m at high tide.

The fouling community is reported to be diverse, with the greatest challenge in July/August. Because of the mild climate, the fouling season extends from April to October. Particularly abundant fouling organisms include slimes (Acananthes and Amphora spp.), algae/weeds (*Enteromorpha* and *Ectocarpus* spp.), barnacles (*Eliminus modestus* and *Semibalanus balanoides*), molluscs (*Mytilus edulis*) and hydroids (*Tubularia* spp.). Additional organisms include polyzoans, tubeworms (Serpulids), sponges (*Halichondria* spp.) and tunicates (*Botyllus* spp.).

UK test site 2

This is located in temperate waters on an estuary. Typical water temperatures, at UK test site 2, range from a winter low of about 8 ^{o}C in February/March to a summer high of about 20 ^{o}C in August. Salinity is generally in the range of 32-34 % but may on occasion drop below 30 % immediately after heavy rainfall. The sea water pH is generally in the range 8.10 - 8.25.

A diverse range of fouling challenge is present at the site, particularly abundant fouling include slimes (*Acananthes* and *Amphora* species), barnacles (*E. modestus*), molluscs (*M. edulis*), amphoids (Jassa) and hydroids (*Tubularia* sp.). Additional organisms include algae/weeds (*Enteromorpha* and *Ectocarpus* sp.), polyzoans, tubeworms (serpulids), sponges (*Halichondria* sp.) and tunicates (sea squirts). The fouling challenge is seasonal and is characterised by a severe barnacle challenge from about May until about September.

Singapore test site

The Singapore test site is located in tropical coastal waters. Typical water temperatures range from about 25 - 30 ^{o}C with little seasonal variation. Salinity is generally about 35 % and sea water pH is generally within the range 8.00-8.25.

Fouling is characterised by an extremely heavy challenge from barnacles, molluscs, tubeworms, polyzoa, slime and felt which persists throughout the year with only slight seasonality.

Fouling of the test panels is assessed visually by the amount and type of each organism. Six main types of fouling are recognised: slime; adherent slime; brown felt; weed; barnacles; hydroids.

For each of these fouling types a subjective visual assessment is made and scored on a scale ranging from 0 to 3 in steps of 0.5. The scoring system is shown in Table 6.2.1.

Table 6.2.1 : Subjective Visual Assessment Scoring System

subjective fouling	fouling score
nil	0
trace	0.5
slight	1
slight to moderate	1.5
moderate	2
moderate to heavy	2.5
heavy	3

Each visual score is then converted to a numerical rating by multiplication by an appropriate factor which is dependent on the type and importance attached to the differing fouling types. The factors are outlined below in Table 6.2.2. In completing an assessment the numerical ratings for each fouling type are added together and the result subtracted from 100 to give an overall antifouling rating for the coating under test. A completely clean panel would give an antifouling rating of 100, and a completely fouled panel an antifouling rating of 0.

Table 6.2.2 : Weighting Factors Applied To The Different Types Of Fouling

type of fouling	weighting or multiplication factor
slime	8
adherent slime	24
brown felt	24
weed	32
barnacles	32
hydroids	32
tubeworms	32
bryozoans	32

Full details of scoring and assessment are provided for each fouling type. Photographs of the test panels taken at the end of the study are also provided to the HSE.

SECTION 3: RAFT TEST METHOD 3

Full details of the study protocols have not been provided. Panels measuring 200 x 400 mm are coated with the test product and immersed in sea water on a raft for a period of time. A blank test panel serves as a negative control. Fouling of the panels is assessed periodically.

Singapore test site

The study site in Singapore is considered representative of tropical waters, with temperatures of 25 to 30 ^{o}C, and heavy fouling.

Norwegian test site

The Norwegian test site is considered representative of temperate waters with temperatures of 0 - 20 ^{0}C and a broad spectrum of fouling organisms.

UK test site

is considered representative of temperate waters, with temperatures of 0 to 20 ^{o}C, and broad spectrum fouling. Because of the presence of excess nutrients (pollution), algal fouling is reported as heavy.

USA test site

is considered representative for 'tropical' waters, with temperatures of 25 to 30 ^{o}C, and heavy fouling.

The fouling is classified as follows:

Light slime - bacteria, microalgae and protozoans which were easily removed from the test panel.

Dense slime - the above organisms, but removal from the panel was not easy.

Weed - green, red and brown algae.

Animals - barnacles, tubeworms, mussels, hydroids and bryozoa.

The extent of fouling ie. trace, slight, medium or heavy, is given a rating according to the type and degree of fouling. These weightings are presented in Table 6.3.1.

Table 6.3.1. : Weighting System Used By The Company For Assessing The Degree Of Fouling In Raft Tests

fouling organisms	assessment (%)			
	trace	slight	medium	heavy
light slime	0	1	3	5
dense slime	3	5	10	20
weed	5	10	30	50
animals	5	10	30	50

The amount of fouling is calculated by adding up the weightings and then subtracting from 100. Zero growth then gives a rating of 100 (100-0), and heavy fouling a rating of 0 (100-100). The antifouling performance is evaluated as shown in Table 6.3.2.

Table 6.3.2 : Antifouling Performance Rating

performance	rating (%)
bad	0-50
poor	50-70
good	70-90
very good	90-98
excellent	98-100