Australian Government

Australian Pesticides and Veterinary Medicines Authority

POLIHEXANIDE CARCINOGENICITY: ANALYSIS OF HUMAN HEALTH RISK

Prepared for the APVMA by the Office of Chemical Safety and Environmental Health, Office of Health Protection, Department of Health and Ageing, Canberra

MAY 2007

(AS AMENDED JUNE 2011)

This document is published by the APVMA. In referencing this document the APVMA should be cited as both author and publisher.

ISBN: 978-0-9870591-6-1

Website: This publication is available from the APVMA website: http://www.apvma.gov.au

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PREFACE: This document contains an evaluation of the toxicokinetics and toxicology of polihexanide, focussing on its potential carcinogenicity.

ABBREVIATIONS

TIME

d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

WEIGHT

bw	Body weight
g	Gram
kg	Kilogram
µg	Microgram
mg	Milligram
ng	Nanogram
wt	Weight

LENGTH

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

DOSING

id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day

VOLUME

L	Litre
mL	Millilitre
µL	Microlitre

CONCENTRATION

M	Molar
ppb	Parts per billion
ppm	Parts per million

CLINICAL CHEMISTRY, HAEMATOLOGY

ALT	alanine aminotransferase
ALP	alkaline phosphatase
AST	aspartate aminotransferase

TERMINOLOGY

GI	Gastrointestinal
GLP	Good Laboratory Practice
MWtF	Molecular weight fraction
NOEL	No Observed Effect Level
QA	Quality Assurance

ORGANISATIONS & PUBLICATIONS

APVMA	Australian Pesticides and Veterinary Medicines Authority
DoHA	Department of Health and Ageing
EU	European Union
FAISD	First Aid Instructions & Safety Directions
NICNAS	National Industrial Chemical (Notification and Assessment) Scheme
OCSEH	Office of Chemical Safety and Environmental Health
OECD	Organization for Economic Cooperation and Development
TGA	Therapeutic Goods Administration
US EPA	United States Environmental Protection Agency

EXECUTIVE SUMMARY

Polihexanide is a polymer of chlorhexidine that is used as an antimicrobial for the control of microorganisms, algae and fungi in swimming pools and spas. It is also used as a disinfectant in veterinary products, and as a sanitiser for milk handling equipment.

Polihexanide is also used in non-agricultural/veterinary situations which are outside of the APVMA's regulatory jurisdiction. It is used as a biocide (disinfectant) in medical equipment, medical procedures, contact lens cleaners, food preparation surfaces, and industrial situations. These uses are regulated by the Therapeutic Goods Administration (TGA) and the National Industrial Chemical (Notification and Assessment) Scheme (NICNAS) within the Department of Health and Ageing.

The reconsideration of registrations of products containing polihexanide and associated label approvals began in July 2005 based on concerns over chemistry, toxicology, occupational health and safety, and residues. Polihexanide was nominated because of concerns related to the potential for it to be a carcinogen in humans through either dermal and/or oral exposure. The Office of Chemical Safety and Environmental Health (OCSEH) identified these concerns following a review of the first aid instructions and safety directions (FAISD) undertaken at the request of the APVMA.

The OCSEH has completed a toxicological hazard assessment of potential carcinogenicity for polihexanide. The assessment identified polihexanide as a potential carcinogen in whole-of life studies in rodents via the oral route but only at high exposure levels which are quite unlikely to be encountered in occupational or public settings. Negative results were obtained in an 80-week dermal study in mice. There were no carcinogenic effects on skin. The occurrence of haemangiosarcoma in the liver at the high dose in the dermal study was considered not to be treatment related as it was within historical controls. Since polihexanide did not appear to be genotoxic and clear NOELs were demonstrated in animal carcinogenicity studies, the OCSEH does not regard carcinogenicity findings in rodents as a barrier to continuing registration of products containing polihexanide.

The APVMA will continue to address the remaining components of the polihexanide review as detailed in the scope document, including addressing the remaining toxicological and occupational exposure elements with the establishment of appropriate public health standards and first aid instructions and safety directions. The detail of these assessments will be published in a subsequent report.

TOXICOLOGICAL ASSESSMENT

TOXICOLOGY HAZARD PROFILE

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS	
Rate and extent of oral absorption	<10% in rats
Distribution	Limited data
Potential for accumulation	No data
Rate and extent of excretion (rat)	Majority excreted in 24 hours, mostly in faeces (> 90%) and a low amount in urine (< 10%) and bile (0.2%).
METABOLISM	No data
Toxicologically significant compounds (animals, plants and environment)	Polihexanide
SHORT-TERM TOXICITY	
Target/critical effect	Systemic toxicity (6 months dog study): histopathological alterations in liver and kidneys (bile stasis, focal hepatocellular degeneration and necrosis, and focal proximal tubular nephrosis)
Lowest relevant oral NOEL (mg/kg bw/d)	100 ppm in dogs (approx. 2.5 mg/kg bw/d)
Lowest relevant dermal NOEL (mg/kg bw/d)	Not available.
Lowest relevant inhalation NOEC (mg/m^3)	Not available.
GENOTOXICITY	Non-genotoxic
LONG-TERM TOXICITY AND CARCINOGENICITY	
Target/critical effect	Haemangioma and haemangiosarcoma in the liver of rats; effects on the liver and recto-anal junction in mice (haemangiosarcoma in the liver and squamous cell carcinoma at the recto-anal junction at the highest dose).
Lowest relevant NOEL (mg/kg bw/d)	36 mg/kg bw/d in rat two-year dietary study; 55 mg/kg bw/d in mouse two-year dietary study.
CARCINOGENICITY	Oncogenicity at high doses in chronic mouse and rat studies.

SUMMARY TOXICOLOGY REPORT

Metabolism and Toxicokinetics

The bioavailability of polihexanide was investigated in Sprague-Dawley rats (5 Wistar-derived rats [Alpk:APfSD]/sex/group) that were pre-fed diets containing 200 ppm or 2000 ppm polihexanide (10 and 100 mg/kg bw nominal dose) for 14 days followed by a single radiolabelled dose administered via gavage (either 0.08 mg/kg bw or 0.8 mg/kg bw). The study was Good Laboratory Practice (GLP) and Quality Assurance (QA) compliant but compliance with OECD test guidelines was not indicated. Low and high oral doses resulted in similar patterns of excretion in both sexes, with the entire administered dose excreted within 72 hours. The faeces represented the major route of excretion (105–109% of the administered dose) in all cases, with the majority occurring within 24 hours of dosing. Urinary excretion was low in all cases (2–3% of the administered dose), with the majority occurring within 24 hours of dosing. The overall recovery of administered dose in tissue and carcass was low (0.42–2.2%) and was similar for both sexes. The levels in whole blood and plasma were insignificant 72 hours post-dose. The mean percentage of administered polihexanide absorbed at the low dose was 4.7% and 3.9% for males and females respectively; the corresponding percentages for the high dose were 3.0% and 2.6% respectively. A slightly higher absorption occurred in males at both doses (Lythgoe & Howard, 1995).

Following a single oral dose of 20 mg/kg bw polihexanide (as Vantocil 1B), absorption was low in rats (only 2.6–7.8% of the administered dose), and the unabsorbed dose was eliminated in the faeces. Excretion in bile was low (<0.2%). The absorption of low molecular weight fractions (MWtF) of polihexanide components was greater than that of mid and high MWtF components. The absorption of low MWtF components was higher in male rats than in female rats. A total of 0.53–0.74% of the administered dose was detected in all collected tissues three days after dosing, with the highest level measured in the liver (0.18–0.19% of the administered dose). Attempts to identify the metabolites were not successful (Lythgoe *et al.,* 1995).

In another study, following a single oral dose of 20 mg/kg bw polihexanide (as Vantocil 1B) in male rats, 93%, 6%, 0.6% and 0.2% were excreted in faeces, urine, bile and expired air, respectively, indicating poor absorption (<7%) from the gut. Urinary components contained mainly low MWtF, suggesting that the absorption of low MWtF was greater. In a part of the study involving dietary administration of polihexanide to male rats over five weeks, the maximum concentration in fat reached 1.2 ppm, 0.6 ppm in the liver, 0.8 ppm in kidney, 0.1 ppm in the heart, with undetectable levels in the brain (Bratt, 1975).

Subchronic Studies

In a 90-day rat study (25 Wistar rats/sex/dose), Antibacterial 9073 (25% aqueous solution of polihexanide) was administered via a diet containing 0, 2500, 5000 ppm (equal to 0, 625, 1250 ppm or approximately 0, 30 and 60 mg/kg bw/d polihexanide respectively). The study is relatively old. No GLP/QA statements or test guidelines were given. The study included a more limited array of test parameters than is recommended by the OECD Test Guideline 408: Repeat-dose 90-day oral toxicity study in rodents. At a dose of 5000 ppm, there was a significant decrease in body weight gain in both sexes (13% males, 17% females) and this was associated with decreased food consumption over the duration of the study. Five of 20 females in the 5000 ppm dose group showed a slight to moderate degree of haemosiderin deposition in liver and Kupffer cells. This histopathological finding was not noted in the control animals and thus was considered treatment-related. No histopathological information for the 2500 ppm dose was provided in the study and this was considered to be a significant data deficiency. Inclusion of a broader array of haematological parameters in

this investigation would have enabled further interpretation of the effect. This study is considered to be of limited value for regulatory purposes due to a lack of histopathological information at 2500 ppm and inadequate monitoring of haematology (Griffiths *et al*., 1966a).

In a 90-day dog study (4 beagle dogs/sex/dose), Antibacterial 9073 (25% aqueous solution of polihexanide) was administered via a diet containing 0, 5500, 11000 ppm (equal to 0, 1375, 2750 ppm or approximately 0, 34 and 69 mg/kg bw/d polihexanide respectively). This study is relatively old. No GLP/QA statements or test guidelines were given. The study included a more limited array of test parameters than is recommended according to OECD Test Guideline 409: Repeat-dose 90-day oral toxicity study in non-rodents. Decreases in body weight gain were noted in females at 5500 ppm and both sexes at 11000 ppm compared to controls but were not considered toxicologically significant since the overall weight differences from control animals were small (<10%). Haemosiderin deposition of minimal to slight degree was present in the spleen of males and females dosed at 11000 ppm and females given 5500 ppm, and was considered to be treatment-related. The NOEL for systemic toxicity following administration of Antibacterial 9073 was below 5500 ppm (equal to 1375 ppm of polihexanide or approximately 34 mg/kg bw/d) based on an increased incidence of haemosiderin deposition in the spleen at all doses (Griffiths *et al*., 1966b).

In a 26-week dog study (4 beagle dogs/sex/dose), a 20% aqueous solution of polihexanide was administered via the diet at 0, 500, 1500 or 4500 ppm (equal to 0, 100, 300, 900 ppm or approximately 0, 2.5, 7.5 and 23 mg/kg bw/d of polihexanide). The following treatment-related signs were noted at the highest dose of 4500 ppm: decreased bodyweight gain (>10%) with no corresponding decrease in food consumption; clinical chemistry indicators of liver damage (statistically significant *versus* controls, $p<0.05$) including increased aspartate aminotransferase (AST), alanine aminotransferase (ALT) (weeks 13–26), increased bromosulfophthalein retention (weeks 22 and 26) and decreased electrophoretic alpha 2 fraction (weeks 4, 13, 26); increased relative liver and kidney weights (>10%) . Bile stasis, both canalicular and cholangiolar, was noted in 1/4 females at 1500 ppm, 3/4 males and females at 4500 ppm. Varying degrees and forms of hepatocellular degeneration and necrosis were observed at doses of 1500 ppm and above. Kidney lesions of epithelial degeneration or nephrosis noted in 2/4 females at 1500 ppm, 3/4 females at 4500 ppm and 2/4 males at 4500 ppm were very mild, focal and involved primarily the proximal convoluted tubules. The NOEL was approximately 2.5 mg/kg bw/d, based on histopathological alterations in the liver and kidneys (bile stasis, focal hepatocellular degeneration and necrosis, and focal proximal tubular nephrosis) at higher doses (Trutter & Patterson, 1977).

Chronic Studies

Oral exposure

In a one-year dog study (4 beagle dogs/sex/dose), a 20% aqueous solution of polihexanide was administered via the diet at 0, 300, 1500 or 3000 ppm polihexanide (equivalent to 0, 9, 46 and 91 mg/kg bw/d for males and 0, 9, 45 and 91 mg/kg bw/d for females). The original high dose level of 4500 ppm was reduced to 3000 ppm from day 1 of week 11 or 12 of treatment due to excessive treatment-related effects. Three of 4 males and one out of 4 females receiving 4500 ppm/3000 ppm were killed prematurely during the study after displaying severe and unexpected clinical signs of toxicity. This included marked reddening/peeling of scrotal skin, lack of appetite and bodyweight loss and/or indications of hepatotoxicity (elevated plasma ALT and AST). A marked increase in plasma ALT was observed in surviving animals at a dose of 4500 ppm/3000 ppm. Cholesterol values in surviving female dogs were statistically significantly decreased ($p<0.01$). Dermatitis, featuring necrosis, parakeratosis and micro-abscess formation, was

observed on the limbs and/or scrotum of 2 males and on the limbs and chin of one female that were sacrificed prematurely at a dose level of 4500 ppm/3000 ppm. A decrease in liver weight was observed in the surviving male at 3000 ppm. Microscopic changes in the liver of both sexes at this dose included the presence of large eosinophilic intracytoplasmic inclusion bodies in centrilobular and mid-zonal hepatocytes, and to a lesser extent cellular swelling and single cell necrosis. Bilateral testicular tubular degeneration was observed in 2/4 males. This resulted in a low testis weight for the surviving animal and was accompanied by Leydig cell hyperplasia. The NOEL was 1500 ppm (45 mg/kg bw/d) based on clinical chemistry changes, decreased liver and testis weights in males and accompanying histopathological alterations at the highest dose (3000 ppm) (Horner, 1995).

Chronic Studies – Carcinogenicity Studies

Oral exposure

In a two-year mouse study (55 animals/sex/dose), a 20% aqueous solution of polihexanide was administered via the diet at 0, 400, 1200 or 4000 ppm of polihexanide (equivalent to 0, 54.7, 167 and 715 mg/kg bw/d of polihexanide in males and 0, 69, 216.5 and 855.5.3 mg/kg bw/d for females). The survival rate was comparable among all male groups including the control group. In females, the survival rate at 4000 ppm was 12% lower than controls at study termination. Anal swelling and discharge were the main treatment-related clinical observations at 4000 ppm. At 4000 ppm, bodyweight gain was severely reduced in both sexes during treatment compared to control animals despite an increase in food consumption. An increased incidence of masses in both sexes at 4000 ppm correlated with increased incidences of haemangiosarcoma in the liver. Non-neoplastic lesions such as hepatocyte hypertrophy, induction of hepatic DNA synthesis (increased ploidy) and increased pigmentation (lipofuscin and haemosiderin) were observed in both sexes at 1200 and 4000 ppm. Gross examinations also revealed an increased incidence of prolapsed and/or swollen anus in both sexes at 4000 ppm. This was correlated with a dose-related increased incidence of inflammation and squamous cell hyperplasia around the recto-anal junction at 1200 and 4000 ppm. There was also an increased incidence of squamous cell carcinoma in the same area in both sexes at 4000 ppm. Adenocarcinoma at the recto-anal junction was also observed in 1/49 males at 4000 ppm. Increased incidences of luminal dilatation and epithelial hyperplasia were observed in the gall bladder in both sexes at 4000 ppm. Also at this dose, there was an increased incidence of extramedullary haemopoiesis in the spleen. The NOEL was 400 ppm (equivalent to approximately 55 mg/kg bw/d) based on toxicity in the liver and the recto-anal junction at the next highest dose (Milburn, 1996).

In a two-year rat study (64 animals/sex/dose), a 20% aqueous solution of polihexanide was administered via the diet at 0, 200, 600 or 2000 ppm of polihexanide (equivalent to 0, 12.1, 36.3 and 126.1 mg/kg bw/d of polihexanide in males and 0, 14.9, 45.3 and 162.3 mg/kg bw/d for females). The survival rate was comparable among all male groups. In females, the survival rate was 13% lower in the 2000 ppm group than in the controls. There were no overt signs of toxicity or abnormal behaviour observed during the study. Female rats fed 2000 ppm polihexanide had a decrease in bodyweight gain compared to control animals. Food consumption and food conversion efficiency, however, was not affected by treatment. Plasma ALP activity was increased in female rats at 2000 ppm during the study. Histologically, haemangioma in the liver was observed in 2/64 males and 2/64 females at 2000 ppm. Haemangiosarcoma in the liver was observed in 1/64 females at 2000 ppm. The NOEL was 600 ppm (equivalent to approximately 36 mg/kg bw/d), based on decreased bodyweight gain, increased ALP activity and the presence of haemangioma and haemangiosarcoma in the liver at the highest dose (Horner, 1996).

Dermal Exposure

In a mouse study, polihexanide was applied to the clipped dorsal skin at dose levels of 0, 0.6, 6.0 or 30 mg/mouse/d for five days per week over an 80-week period. Survival rates in both sexes at 30 mg/mouse were lower than in other groups. Clinical signs such as irritation of the skin, hyperkeratosis and desquamation and bilateral protrusion of the eyes were restricted to the highest dose. Bodyweight gain in the highest dose group was reduced during the study compared with control animals. Haemangiosarcoma in the liver was observed in 2/50 females at 30 mg/mouse but none in the other groups. This was not considered to be related to treatment because the incidence was within the historical control range (Ishmael and Weight, 1978; Clapp, 1990). Polihexanide was not considered to be a carcinogen in mice in this study when dermally applied (Clapp, 1990).

Genotoxicity Studies

Polihexanide (20%) was not mutagenic to bacteria (Callander, 1989) or clastogenic to human lymphocytes (Howard, 1989). Polihexanide (20%) did not cause unscheduled DNA synthesis in rat hepatocytes (Trueman, 1989) and was negative in the *in-vivo* micronucleus test in mice (Randall & Beck, 1989).

HAZARD ASSESSMENT

Discussion

Introduction

The current review of polihexanide was undertaken under the auspices of the APVMA's Chemical Review Program because of concerns related to potential carcinogenicity.

Adequacy of data

The evaluated studies were conducted between 1966–96 and most did not comply with GLP or QA, except for a number of the more recent studies, including the carcinogenicity studies. No test guidelines were cited for any study. The toxicokinetics studies were of limited value. Metabolism studies, short-term and subchronic studies in mice were not provided. Although the same regulatory weight as for more modern study counterparts cannot be placed on some of the older studies, all studies except one were considered to be acceptable for regulatory purposes. The 90-day rat study was considered unacceptable for regulatory purposes because there was no histopathological information provided for the mid-dose level of 2500 ppm and haematological parameters were inadequately monitored.

Toxicokinetics

The oral bioavailability of polihexanide was only 4–5% in rats, which excreted 90% of an oral dose of polihexanide via the faeces with very little biliary excretion. Very small quantities were excreted in the urine. There were no *in vitro* and *in vivo* percutaneous absorption studies available for evaluation. Therefore, the extent of dermal absorption of polihexanide is not known.

Genotoxicity

There was no evidence that polihexanide is genotoxic *in vitro* or *in vivo*.

Carcinogenicity

In mice, polihexanide caused an increased incidence of haemangiosarcoma in the liver and squamous cell carcinoma at the recto-anal junction at high exposure levels (approximately 700–850 mg/kg bw/d) with the oral NOEL for carcinogenicity being approximately 167 mg/kg bw/d. Squamous cell carcinoma was considered to be a consequence of chronic irritation and subsequent inflammation at the recto-anal junction although it is worth noting that tissue iron deposition may be a factor in the development of colorectal carcinoma in humans (Labropoulou *et al.,* 2004). In the rat chronic study, one (female) rat out of 128 was found to have haemangiosarcoma in the liver and 4/four rats out of 128 had haemangioma in the liver at doses of approximately 120–160 mg/kg bw/d. It is possible that this was treatment-related since these tumours are rare in this strain of rats. However, an expert peer review concluded that the tumours were only sporadic occurrences and not related to treatment, given the absence of an incidence of preneoplastic findings, for example (Busey, 1996).

In mice, the highest incidence of treatment-related tumours was liver haemangiosarcoma which develops from the endothelial cell component of the vascular sinusoidal cells of the liver. In humans, it is a rare

malignant liver tumour and constitutes only 2% of all primary tumours of the liver. Epidemiological data collected from 1975–87 in England and Wales showed that there was an annual incidence of 1.4 cases per 10 million people. Haemangiosarcoma in the liver in humans had been associated mainly with exposure to vinyl chloride and, to a much lesser extent, arsenic (Kielhorn *et al.,* 2000). The genotoxic mode of action underlying the development of haemangiosarcoma in the liver by vinyl chloride is well established (Bolt, 2005; Dogliotti, 2006) but since polihexanide has not shown any evidence of genotoxic activity, it is unlikely that a similar mechanism exists for polihexanide.

The industrial chemical 2-butoxyethanol has also been reported to induce liver haemangiosarcoma in male mice but not in female mice, rats or humans (Klaunig and Kamendulis, 2005). Data indicate that 2-butoxyethanol causes red blood cell haemolysis and haemosiderin build-up in the Kupffer cells of male mice after just 13 weeks of exposure. That is, there is an accumulation of haemosiderin (iron) in the phagocytic Kupffer cells of the liver. Increased iron levels associated with 2-butoxyethanol-induced haemolysis produces liver oxidative damage and increased DNA synthesis in both endothelial cells and hepatocytes. It has been hypothesised that these events can contribute to the transformation of the endothelial cells to haemangiosarcomas in male mice.

On the other hand, in the current case of polihexanide, the haemangiosarcomas occurring in the chronic mouse study were not associated with such liver effects. This may have been because individuals that developed liver haemangiosarcoma died before the histological changes in the liver seen in other individuals had time to develop. It is possible that polihexanide is capable of causing haemangiosarcomas in the liver as an independent effect from the other effects on the liver. Another possibility is that the haemangiosarcomas observed at the high dose in male and female mice were not treatment related but were a chance occurrence of sporadic tumours somewhat above historical control levels, as argued for the cases in rats (Busey, 1996).

In any case, the evidence of liver tumours and squamous cell carcinoma at the recto-anal junction in rodents is of some interest from a human health point of view. However it should be noted that polihexanide is associated with cancer in rodents only at high doses which are unlikely to be encountered in occupational or public settings, it does not appear to be genotoxic, and clear NOELs were demonstrated in animal carcinogenicity studies. Therefore the OCSEH does not regard the observed tumours as a barrier to continuing registration of products containing polihexanide.

MAIN TOXICOLOGY REPORT

1 INTRODUCTION

Polihexanide is a polymer of chlorhexidine that is used as an antimicrobial for the control of micro-organisms, algae and fungi in swimming pools and spas. It is also used as a disinfectant in veterinary products, and as a sanitiser for milk handling equipment. In addition to the Agvet (agricultural and veterinary) uses described, polihexanide is also used in non-Agvet situations which are outside the APVMA's jurisdiction. These include biocide (disinfectant) uses in medical equipment, medical procedures, contact lens cleaners, food preparation surfaces, and industrial situations.

Polihexanide was nominated for toxicological review because of concerns related to potential carcinogenicity.

1.1 Chemistry - Technical Active

Common name: Polihexanide

Chemical name: Poly(hexamethylene biguanide) hydrochloride

Trade names/synonyms: A-Breeze, Baquacil, Baquacil Ultra, Caswell No. 676, Chlorhexidine complex, Cosmoquil QC, PHMB, Polyhexanide, Vantocil 1B, Vantocil P (20% w/w polihexanide)

CAS Registry numbers: 27083-27-8

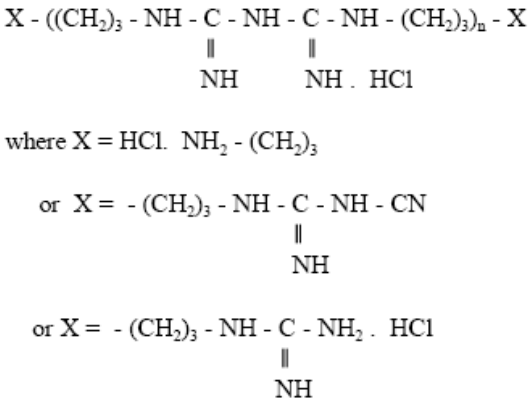
32289-58-0

Empirical formula: $(C_8H_{17}N_5.\ HCl)_{n+1}$ n = 1 - 40, average n value is between 10–13

Chemical structure:

The position of the radiolabel (^{14}C) is ind

Chlorhexidine



Chemical and physical properties

Colour:	Very faint yellow
Physical state:	Mobile liquid
Melting point:	Does not melt; decomposition onset 205–10°C
pH of 5% solution:	5.7
Specific gravity:	1.04 at 20°C
K_{ow}:	2.3 x 10^{-3}
Boiling point :	100°C
Solubility in water:	40 g/100 g solution
Stability:	14 days at 54 ± 2°C

Technical active - Declaration of Composition and Batch Analysis

A declaration of composition for technical grade polihexanide was not provided.

1.2 Products

At the time of preparation for this report there were seven registrants for polihexanide in Australia and nine registered products.

2 METABOLISM AND TOXICOKINETICS

Lythgoe RE & Howard EF (1995) PHMB: Bioavailability Following Dietary Administration in the Rat. Zeneca Central Toxicology Laboratory. Alderley Park, Macclesfield, Chesire, UK. Report No. CTL/P/4595. Lab Project No. URO 468. Report date: March 22, 1995. Study period: October 1994–January 1995.

GLP/QA: Yes.
Guidelines: No.

Methods

The bioavailability of polihexanide was investigated in male and female Sprague-Dawley rats (5/sex/group; Wistar-derived Alpk:APfSD strain; approx 7–9 weeks of age; 208–338 g; BSS, Alderly Park, Alderly Edge, Macclesfield, Cheshire UK) pre-fed diets of 200 ppm or 2000 ppm polihexanide (10 and 100 mg/kg nominal dose) for 14 days followed by a single radiolabelled dose administered via gavage. Animals pre-fed at the lower dose were given a single dose of 0.08 mg/kg bw (0.2 MBq/kg). Animals pre-fed at the higher dose were given a single dose of 0.8 mg/kg bw (2 MBq/kg). A 9% suspension of [^{14}C]-polihexanide (CTL Reference Y00156/009; specific activity 1.85 GBq/4 mmol hexamethylene diamine) was mixed with a vehicle of sterilised, double deionised water and gum tragacanth (1%) prior to dosing. Clinical observations were not carried out during this study. After the radiolabelled dose the animals were kept in metabolism cages for the collection of excreta and once again fed with diet containing unlabelled polihexanide at the same dose levels (either 200 or 2000 ppm). Urine and faeces were separately collected at 24 hourly intervals until 72 hours after radiolabelled dosing, the end of the study. Cage rinses were collected together with urine at each sample point. A final cage wash was also retained for analysis. At the end of the study, rats were killed by exsanguination by cardiac puncture and a sample of blood was collected in heparin vials for analysis. One portion of blood was used to separate the plasma. The gastrointestinal tract and contents, and residual carcass were also retained for analysis. Oxidised and non-oxidised samples were radioassayed by liquid scintillation counting. Analysis of unlabelled and labelled polihexanide was also carried out by Size Exclusion Chromatography.

Results

High overall recoveries (approximately 110% of calculated dose) were observed in this study, attributable to the high proportion of recovered radioactivity found in some individual samples.

Excretion: The results for the excretion of radioactivity in urine (including cage washes) and faeces are presented as group mean results in Table 1. Low or high oral doses resulted in similar patterns of excretion of radioactivity in both sexes, with the total administered dose being excreted within 72 hours. In all cases, the faeces represented the major route of excretion, nominally accounting for 105–109% of the administered dose. The majority of faecal excretion occurred within 24 hours of dosing and was complete within 72 hours. Urinary excretion was low and together with cage wash accounted for approximately 2% of the administered dose. The majority of urinary excretion occurred within 24 hours of dosing. Excretion of radioactivity in the expired air was not measured in this study.

Table 1: Mean excretion of radioactivity within 72 hours (% of dose; mean±Sprague-Dawley; n=5/sex)

DOSE (MG/KG BW)	SEX	SAMPLE	0-24 H	24–48 H	48–72 H	0–72 H	FINAL CAGE WASH	TOTAL EXCRETED
0.08	Male	Urine	1.88±0.35	0.17±0.07	0.09±0.03	2.14±0.32	1.29±0.89	108.43±2.64
		Faeces	91.65±4.69	12.16±4.19	1.20±0.37	105.00±2.48		
	Female	Urine	1.90±0.36	0.15±0.02	0.14±0.05	2.19±0.32	0.53±0.17	112.10±5.59
		Faeces	98.78±4.46	9.78±4.65	0.83±0.31	109.38±5.83		
0.8	Male	Urine	1.92±0.13	0.22±0.07	0.11±0.04	2.26±0.15	0.48±0.07	108.95±1.78
		Faeces	90.85±4.76	14.22±4.31	1.15±0.37	106.21±1.77		
	Female	Urine	1.33±0.52	0.25±0.10	0.17±0.05	1.76±0.46	0.58±0.17	106.98±2.31
		Faeces	89.83±6.71	13.15±6.36	1.65±1.30	104.63±2.15		

Tissue Distribution: Table 2 presents the mean tissue and carcass retention of radiolabel at 72 hours following dietary administration of polihexanide, expressed as a percentage of the applied dose. Low levels of radiolabel were found in the gastrointestinal GI tract and contents, and the carcass. As a proportion of the administered dose, the recovery of radioactivity was relatively higher at the low dose than at the high dose and was similar for both sexes. The levels in blood 72 hours post-dose were similar for both dose levels ranging between 0.001 and 0.003 μg equiv/g. Plasma levels were lower than those found in whole blood, being lower than the limit of detection in all samples.

Table 2: Retention of radioactivity in tissue and carcass at 72 hours (% of dose)

DOSE (MG/KG BW)	SEX	GI TRACT	GI CONTENTS	CARCASS	TOTAL
0.08	Male	0.11	0.80	1.30	2.20
	Female	0.10	0.92	1.142	2.16
0.8	Male	0.02	0.15	0.25	0.42
	Female	0.02	0.19	0.22	0.43

n=5/sex

Bioavailability: The absorbed dose (or bioavailable dose) in this study was calculated as the amount of dose excreted in urine plus that present in the carcass following removal of GI tract and its contents. The dose recovered in cage washings has been summed with urine as the majority of the material was considered to be of urinary origin. It was previously established (Lythgoe *et al*., 1995) that the polihexanide dose recovered in faeces following a single oral exposure represents unabsorbed test substance. In that study, only a very minor proportion of the absorbed dose was eliminated via bile to be returned to the GI tract. Therefore, in this study the unabsorbed dose was calculated as the amount of dose excreted in the faeces together with the contents of the GI tract after removal. The mean percentages of administered polihexanide absorbed

following the low dose were 4.7% and 3.9% for males and females, respectively. At the high dose, the corresponding percentages were 3.0% and 2.6%, respectively. Thus, a slightly higher absorption was apparent in males at both doses.

Bratt, H (1975) Vantocil IB: Absorption and excretion studies in the rat (interim report). Zeneca Central Toxicology Laboratory, Macclesfield, UK. Report No. CTL/P/163B. Report date: March 1975.

Test compound: ^{14}C-labeled (4.6 µCi) Vantocil IB (containing 20% Polihexanide)
Batch: Not specified
Test species: rats (male, but age, bw, species, no. of rats and groups not specified)
GLP: None
Guidelines: none

Absorption and excretion: A single oral dose of ^{14}C-labeled (4.6 µCi) Vantocil IB 100 mg/kg bw (containing 20% polihexanide) was given to male rats. The report stated that Vantocil IB contained at least 10 components but details were not specified. Faeces and urine were collected at 7 time points over 1 to 10 days post-dosing; bile and expired air were also collected (details not specified). The detection method was chromatography

Within 5 days, 93%, 6%, 0.6% and 0.2% of the radiolabel were excreted in faeces, urine, bile and expired air, respectively, indicating poor absorption (<7%) from the gut.

The extracted material in the faeces showed a similar profile to Vantocil IB itself. Chromatography of urinary components showed that they consisted almost entirely of high mobility material largely low MWtF. Vantocil IB itself contains low and high mobility materials, and when these fractions are separated and dosed independently to pairs of rats, 3% and 12% of the low and high mobility material were absorbed, respectively, suggesting greater absorption of low MWtF of Vantocil IB, which was excreted unchanged in urine.

Tissue distribution: A diet containing 100 ppm of ^{14}C-labeled (4.6 µCi) Vantocil IB was administered to male rats for 5 weeks. This was followed by five weeks of normal diet. Rats were sacrificed at weekly intervals, in order to determine tissue retention in abdominal fat, liver, kidney and brain, during treatment and on return to normal diet.

The concentration of polihexanide reached 1.2 ppm in abdominal fat after 3 weeks of administration, which was reduced to 0.3 ppm after five weeks of normal diet. The concentration in liver did not exceed 0.6 ppm during five weeks administration and was reduced to undetectable levels at week 3 after returning to normal diet. The maximum concentrations in kidney, heart and brain were 0.8 ppm, 0.1 ppm, and undetectable, respectively. It was not clear how much food was consumed, therefore, it was not possible to calculate the percentage of dose detected in the tissues.

Lythgoe R.E., Howard E.F., Prescott, E. (1995) PHMB: Absorption, Distribution, Metabolism and Excretion Following Single Oral Dosing (20mg/kg) in the Rat. Zeneca Central Toxicology Laboratory, Macclesfield, UK. Report No. CTL/P/4537. Report date: Feb. 1995

Test compound: ^{14}C-labeled (4.6 µCi) Vantocil IB (containing 20% Polihexanide)
Batch: Not specified
Test species: rats, Alpk:APfSD strain, 163-270g, 6-12 weeks old, 28 males, 13 females, supplied by the Barriered Animal Breeding Unit, Alderley Park, Cheshire.
GLP & QA: Yes
Guidelines: none

A single dose of 20 mg/kg polihexanide was orally administered to rats throughout this study which consisted of 3 experiments. This dose was selected to present a “no effect” dose and the vehicle was sterilised double deionised water. In addition to the unfractionated test substance, the study involved the use of low, medium and high MWtFs of ^{14}C-polihexanide (low, medium and high MWtFs are formed due to oligomerisation and the molecular weight cut-offs were 1k, 3k and 10k, by size exclusion chromatography).

Rats were sacrificed at the end of each experiment, and the following samples were collected: blood, plasma, GI contents, residual carcasses, and tissues. The tissues included brain, liver, kidneys, heart, lungs, spleen, gonads, and representative samples of fat (abdominal), bone (femur) and muscle (femoral). Duplicate samples were analysed.

Experiment 1: Biliary excretion:

A single oral dose of 20 mg/kg ^{14}C-polihexanide was given to bile duct cannulated rats (3/sex). Urine, bile and faeces were collected at 6 time points for 2 days after dosing and radioactivity monitored. No sex differences were observed in excretion profiles. Over 96% of the administered dose was excreted in faeces, <3% in urine, and <0.2% in bile. This indicated poor absorption (about 3% of the administered dose) and low biliary excretion.

Experiment 2: Absorption:

A single oral dose of 20 mg/kg of low, medium and high MWtFs of ^{14}C-polihexanide was each given to 3 groups of male rats (4/group). Urine and faeces were collected at 5–6 time points for 3 days after dosing and radioactivity monitored.

Over 94% of the administered dose was excreted in faeces for all 3 MWtFs, whereas 5.2%, 0.2% and 0.2% was excreted in urine for low, medium and high MWtF, respectively, which indicated greater absorption of the low MWtF. The concentrations in plasma and blood were all less than 0.02 µg equiv/g, and in residual carcasses they were 0.15–0.54% of the administered dose. Total excretions were 100%, 101% and 96%, and total recovery rates (excreta + tissue residues) were 101%, 102% and 96.8% for low, medium and high MWtF, respectively.

Experiment 3: Excretion and distribution:

Low MWtF was selected for this study due to its greater absorption than that of mid and high MWtFs. A single oral dose of 20 mg/kg low MWtF of ^{14}C-polihexanide was given to rats (5/sex). Urine and faeces were

collected at 5–7 time points for 3 days after dosing. Blood, selected tissues and carcasses were also collected.

At 72 hours after dosing, over 93% of the administered dose was excreted in the faeces of both male and female rats (94.1% and 93.5%, respectively). However, a sex difference was observed in urinary excretion (7.8% and 2.6% in male and female, respectively). At 24 hours after dosing, 83–92% of the total absorbed dose was excreted in urine. At 72 hours after dosing, the highest concentrations were in liver (0.57 and 0.75 µg equiv/g, or 0.18% and 0.19% of administered dose, in male and female, respectively) and kidney (0.50 and 0.81 µg equiv/g in male and female, respectively), whereas the residual carcasses contained 0.22% and 0.28% in male and female, respectively. The percentage of the administered dose in kidneys was not provided. Total recovery of administered dose was 102.8% (male) and 97.0% (female).

Attempts to identify metabolites were not successful due to the small amount available in the urine and the poor level of solvent extraction of radioactivity from the faeces.

Conclusion:

Following a single oral dose of 20 mg/kg, polihexanide was poorly absorbed by rats (only 2.6–7.8% of the administered dose), and the unabsorbed fraction was eliminated in faeces (92–97% of administered dose). Less than 0.2% was eliminated in bile. The absorption of low MWtF components of polihexanide was greater than that of mid and high MWtF components, particularly in male rats compared to female rats. Attempts to identify the metabolites were not successful.

3 SUBCHRONIC TOXICITY STUDIES

3.1 Oral

3.1.1 Rats

Griffiths D, Hayes MJ, and McElligott TF (1966a) Ninety-Day Oral Toxicity of Antibacterial 9073 - Albino Rats. Industrial Hygiene Research Labs. Report No. CTL/R/199. Report date: August 1, 1966. Unpublished. Study period: unspecified.

GLP and QA: No.
Guidelines: No.

Materials and Methods

Antibacterial 9073 [a 25% aqueous solution of polihexanide, sample WEM/G/680, no source or compositional analysis supplied] was mixed in the diet and fed to 25 Wistar rats/sex/dose at 0, 2500 or 5000 ppm (0, 625, 1250 ppm polihexanide) for 90 days. There was no rationale for the dose selection.

Treated diets were prepared weekly by mixing the appropriate quantity of test material with diet in water and drying under vacuum. Young adult Wistar rats were sourced from a colony maintained at Alderly Park, Cheshire (acclimatisation period prior to experimentation was not stated). The age of rats at the commencement of dosing was not stated. Males and females weighed between 138–236 g and 122–187 g, respectively, at the start of the study. Rats were housed under standard conditions, with food and water available *ad libitum*.

The frequency of observations for mortality and clinical signs were not stated. Bodyweights and food consumption were recorded weekly. Blood was collected from rats in each group at the start of study and at study termination, just prior to sacrifice. A limited range of haematology parameters were measured. Rats were sacrificed after 90 days by chloroform and an immediate post-mortem examination made. Absolute and relative organ weights were measured from 5/sex/dose group and included: liver, heart, lung, adrenals, kidneys and spleen. The following were examined microscopically: liver, kidney, spleen, heart, lung, adrenals, gonads, thymus, thyroid, pancreas, stomach, duodenum, jejunum, ileum, caecum, colon, salivary gland and mesenteric lymph nodes, spinal cord and brain (cerebrum, cerebellum and pons).

No information regarding statistical analysis methodology was mentioned in this study.

Results

Dietary analysis: No analytical measurements or homogeneity analyses of diets for polihexanide content were performed at either target dose.

Mortalities and clinical signs: There were no deaths or clinical signs.

Body weight and food consumption: Food consumption was decreased at a dose of 5000 ppm throughout the duration of study in both sexes when compared to controls (as much as 13% in males during Week 4). Animals at 2500 ppm also tended to consume less food compared to controls but the difference was small and of doubtful significance, given that there were no associated body weight changes. The decreased food

consumption was attributed to decreased palatability of the diet due to polihexanide. There was a significant reduction in bodyweight gain in the 5000 ppm dose group over the course of the study (13% males, 17% females). This reduction was considered treatment-related due to associated changes in food consumption at the same dose. No significant differences in body weight gain were observed in the 2500 ppm group.

Haematology: There were no treatment-related alterations in the haematological parameters measured.

Pathology: There were no treatment-related gross pathological abnormalities, no changes in organ weights nor organ:body weight ratios.

Histopathology: Five of 20 females in the 5000 ppm dose group had a slight to moderate degree of iron pigment (haemosiderin deposition) in liver and Kupffer cells (Table 3). This was not noted in the control animals. Thus, haemosiderin deposition is considered to be treatment-related. No information on the incidence of this effect at 2500 ppm was provided and this was considered as a major data deficiency. Inclusion of a broader array of haematological parameters in this investigation might have enabled further interpretation of the effect. No other treatment-related histopathological findings were observed.

Table 3: The incidence of haemosiderin deposition*

DOSE (PPM)	MALES		FEMALES	
	0	5000	0	5000
Total examined	20	20	20	20
Liver (haemosiderin in hepatic and Kupffer cells)				
Total affected	0	0	0	5
- Slight degree	0	0	0	1
- Moderate degree	0	0	0	4
Spleen (haemosiderin deposition)				
Total affected	18	17	20	18
- Minimal degree	11	12	1	7
- Slight degree	6	5	10	7
- Moderate degree	1	0	9	4

**no data submitted regarding incidence in the 2500 ppm group.*

Conclusions:

In the absence of histopathological information at the mid dose level of 2500 ppm, a NOEL for haemosiderin deposition in the liver and Kupffer cells cannot be established. Therefore this study is considered to be of limited value for regulatory purposes because of a lack of histopathological information at 2500 ppm and inadequate monitoring of haematological parameters.

3.1.2 Dogs

Griffiths D, Hayes MJ, and McElligott TF (1966b) Ninety-Day Oral Toxicity of Antibacterial 9073- Beagle Dogs. Industrial Hygiene Research Labs. Report No. CTL/R/202. Report date: September 1, 1966. Unpublished. Study period: unspecified.

GLP and QA: No.
Guidelines: No.

Materials and Methods

Antibacterial 9073 [identical to that used in the rat study, section 5.1.1 and prepared in the same manner] was mixed in the diet and fed to 4 beagle dogs/sex/dose at 0, 5500 or 11000 ppm (0, 1375, 2750 ppm polihexanide) for 90 days. There was no rationale for the dose selection.

Beagle dogs used in this study were supplied from an inbred strain maintained at Alderly Park, Cheshire (acclimatisation period prior to experimentation not stated). The age of dogs at the commencement of dosing was not stated. Males and females weighed between 13–14.6 kg and 12.4–14.4 kg, respectively, at the start of the study. Dogs were housed individually under standard conditions, with water available *ad libitum* and food offered twice daily.

The frequency of observations for mortality and clinical signs were not stated. Bodyweights and food consumption were recorded weekly. Blood was collected from each group at the start of study and at study termination, just prior to sacrifice. The following haematology, clinical chemistry and urinalysis parameters were measured: haemoglobin, haematocrit, total white blood cell count, differential white blood cell count, blood urea, serum alkaline phosphatase, liver function test and urinary (pH, specific gravity, glucose, protein, bilirubin, microscopy of centrifuge deposit).

Dogs were sacrificed after 90 days by iv administration of pentobarbitone and an immediate post-mortem examination made. The weight of the following organs was obtained at the time of necropsy: heart, liver, kidneys, adrenals, spleen, thyroid, testicles, epididymis, brain and pituitary. Microscopic examination of the following tissues was made: brain (cerebrum, cerebellum and pons), spinal cord, pituitary, submaxillary gland, thyroid, thymus, heart, lung, aorta, stomach, duodenum, jejunum, ileum, colon, liver, spleen, kidney, bladder, adrenal, ovary and uterus or testis and epididymis and sciatic nerve.

No information regarding statistical analysis methodology was mentioned in this study.

Results

Dietary analysis: No analytical measurements or homogeneity analyses of diets for polihexanide content were performed at either target dose.

Mortalities and clinical signs: There were no deaths or clinical signs.

Bodyweights and food consumption: The study authors claimed that diets were consumed readily. From this it is assumed that there were no treatment-related alterations in food consumption (no food consumption data were provided). Decreases in body weight gain were noted in females at the low-dose and in both

sexes at the high-dose compared to controls (Table 4). These changes were not considered toxicologically significant since the overall weight differences from control animals were all <10%.

Table 4: Overall mean body weight change (%) versus controls

DOSE LEVEL (PPM)	5500	11000
Males	0	- 2.2%
Females	- 4.3%	- 6.5%

n=4

Haematology, clinical chemistry and urinalysis: A reduction in the neutrophil count was observed at 5500 and 11000 ppm in both sexes compared to pre-treatment levels and concurrent controls (Table 5). No statistically significant differences were noted between groups and the study authors noted that all values were within the physiological range (however no historical data was supplied). The finding was not considered treatment-related. All other haematological values measured were unremarkable. Red blood cell counts were not measured.

Table 5: Mean neutrophil counts in dogs following treatment (x10^3 Cmm)

TEST POINT	DOSE (PPM)					
	0		5500		11000	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
Pre-treatment	8.97	6.93	7.78	7.48	7.52	6.74
Terminal	7.61	8.19	4.58	5.48	4.79	4.30

n=4/sex

There were no treatment-related alterations in clinical chemistry or urinalysis parameters.

Pathology: There were no treatment-related macroscopic abnormalities or changes in organ weights or organ:body weight ratios.

Histopathology: Haemosiderin deposition of minimal to slight degree was noted at a higher incidence in the spleen of animals dosed at 11000 ppm and females given 5500 ppm compared to controls, and was considered to be treatment-related (Table 6). Haemosiderosis has been observed in other repeat-dose studies on polihexanide. No other treatment-related histopathologic observations were noted.

Table 6: The incidence of haemosiderin deposition in the spleen

DOSE (PPM)	MALES			FEMALES		
	0	5500	11000	0	5500	11000
Total examined	4	4	4	4	4	4
Spleen						
Total affected	0	0	2	1	3	2

DOSE (PPM)	MALES			FEMALES		
	0	5500	11000	0	5500	11000
- Minimal degree	0	0	0	0	3	0
- Slight degree	0	0	2	1	0	2

Conclusions:

The NOEL in dogs following 90 days of dietary exposure to Antibacterial 9073 was below 5500 ppm (equal to 1375 ppm of polihexanide or approximately 34 mg/kg bw/d) based on an increased incidence of haemosiderin deposition in the spleen at both test doses.

Trutter JA and Patterson DR (1977) 26-week Toxicity Study in Dogs: 20% PHMB. Hazelton Laboratories America Inc. Report No. 458-123. Report date: June 30, 1977. Unpublished. Study duration: September 1976 – March 1977.

GLP and QA: No.
Guidelines: No.

Materials and Methods

Polihexanide [a 20% aqueous solution of polihexanide: IL-780, ADGM 5642, TC#8492, Mix #49/22/122 (first sample); IL-780, ADGM 5642, TC#1126 (second sample); no source or compositional analysis supplied] was mixed in the diet and fed to 4 beagle dogs/sex/dose at 0, 500, 1500 or 4500 ppm (0, 100, 300, 900 ppm of polihexanide) for 26 weeks. There was no rationale for the dose selection. Treated diets were prepared weekly by mixing the appropriate quantity of aqueous test material with control diet. Beagle dogs used in this study were supplied from Hazleton Research Animals, Inc., Cumberland, Virginia (acclimatisation period prior to experimentation was not stated). The age of dogs at the commencement of dosing was 7–8 months. Males and females weighed between 8.1–12.2 kg and 8.5–12.2 kg, respectively, at the start of the study. Dogs were housed individually under standard conditions, with food and water available *ad libitum*.

Observations for mortality and clinical signs were recorded daily. Bodyweights and food consumption were recorded weekly. Blood was collected from overnight-fasted dogs in each group at the start of study (week 0), and at weeks 4, 13 and 26 (study termination, just prior to sacrifice). Haematological parameters tested included haematocrit, haemoglobin, red blood cell count, total and differential white blood cell count. Clinical chemistry parameters included fasting blood sugar, blood urea nitrogen, serum glutamic pyruvic transaminase, alkaline phosphatase, total serum bilirubin, serum glutamic oxaloacetic transaminase, serum potassium, serum chloride, serum calcium, carbon dioxide, total serum protein, serum albumin, serum protein electrophoresis and serum sodium. Overnight urine specimens were collected at the same intervals and the testing included specific gravity, pH, ketones, bilirubin, protein, sugar, and microscopic examination of the sediment.

Due to findings at week 13, the protocol was amended to perform additional blood chemistry studies and blood samples were collected during week 22 for determinations of serum glutamic pyruvic transaminase, alkaline phosphatase, total serum bilirubin, and serum glutamic oxaloacetic transaminase and during weeks 22 and 26 for bromosulphalein retention studies. Ophthalmoscopic examinations were performed on all dogs at weeks 0, 4, 13 and 26. General physical examinations, including heart and lung ausculations, were

performed on two high dose females during weeks 21 and/or 22 and on all dogs during week 26. Based on findings during the study, the protocol was amended and electrocardiographic tracings consisting of 20 second intervals of limb leads I, II, and III were obtained from each dog during week 25.

Dogs were sacrificed after 26 weeks of treatment by exsanguination under anaesthesia and necropsied. The following organ and organ/body weight ratios were determined: pituitary, thyroid, heart, liver, spleen, kidneys, adrenals, testes with epididymides, prostate, ovaries and uterus. Preserved tissues from control and high dose dogs and select gross lesions from low and mid dose dogs were examined microscopically. Tissues included: brain, pituitary, thoracic spinal cord, eyes, mandibular salivary gland, thyroids with parathyroids, thymus, lung, heart, spleen, small intestine (three levels), large intestines, urinary bladder, mesenteric lymph node, gallbladder, liver, kidneys, adrenals, stomach, pancreas, prostate, ovaries, uterus, vagina, bone (rib junction), bone marrow (femoral plug), skeletal muscle, sciatic nerve and any unusual lesions.

Statistical tests chosen for the study were appropriate. Statistical analysis of body weights, food consumption, clinical laboratory data, terminal body weights, organ weights, and organ/body weight ratio data were performed by Bartlett's test for homogeneity of variances and one-way ANOVA. All evaluations were made at the 5% significance level.

Results

Dietary analysis: No analytical measurements or homogeneity analyses of polihexanide content in diets were performed at any dose level.

Mortalities and clinical signs: No treatment-related deaths or clinical signs were noted at 500 or 1500 ppm. One female at 4500 ppm appeared thin and had pale gums at week 19, but was alert and active. Later examinations of this animal showed thinness, a dull hair coat, pale mucous membranes and slow capillary filling. A second female at 4500 ppm also had a thin appearance.

Body weight and food consumption: There were no statistically significant differences in mean weekly body weight and food consumption between test animals and controls. However, the mean body weight gain of the 4500 ppm dose group showed consistent treatment-related decreases with time. The decrease became more pronounced during the last 8 weeks of study, particularly in females, and corresponded with slightly lower than mean control values during this time. Overall mean body weight changes are presented in Table 7. Decreased body weight gain at 4500 ppm was considered to be treatment-related because the magnitude of loss (>10%) in females was considered to be toxicologically significant.

Table 7: Overall mean body weight changes in dogs fed polihexanide during weeks 0-26

DOSE LEVEL (PPM)	0	500	1500	4500
Males	+18%	+12.2%	+18%	-4.8%
Females	+11.7%	+12.5%	8.7%	-15.9%

n=4/sex

Haematology, clinical chemistry and urinalysis: There were no statistically significant differences in haematological values when compared to their respective control values. Treatment-related signs of liver damage were evident in the 4500 ppm dose group for both sexes (Table 8): a statistically significant increase in AST and ALT was observed from weeks 13–26 (45–55% for AST and 65–80% for ALT); increased bromsulphalein retention (measured at week 22 and 26 only) was noted in all animals at week 22 and in 3

females at week 26; and a statistically significant decrease in electrophoretic alpha 2 fraction at weeks 4, 13 and 26 in females. A dose-response pattern was noted for decreased electrophoretic alpha 2 fraction at weeks 13 and 26 but was not statistically significant. Statistically significant changes noted in other clinical chemistry parameters were sporadic in nature and did not indicate any distinct treatment related trends. All other clinical chemistry values were comparable to controls and stated to be within acceptable laboratory limits. Urinalysis findings were unremarkable. No historic control data were supplied.

TABLE 8: SELECTED CLINICAL CHEMISTRY FINDINGS

WEEKS	MALES				FEMALES			
	0	500	1500	4500	0	500	1500	4500
AST								
0	19.7	21.0	19.0	18.7	21.2	18.7	21.5	20.2
4	22.7	20.7	24.0	23.2	19.5	22.2	22.7	22.5
13	25.2	31.5	39.2	45.7**	27.7	27.3	32.7	48.2**
22	27.7	26.7	29.0	29.5	27.2	30.5	27.5	50.2
26	28.0	27.0	31.7	62.2**	27.0	31.2	32.7	57.2**
ALT								
0	22.0	25.5	20.2	20.7	23.5	24.7	23.0	21.0
4	30.7	33.2	28.5	30.0	26.0	27.5	30.5	28.7
13	27.2	30.2	27.7	79.5**	23.7	24.0	30.2	170.5**
22	33.7	36.5	31.5	232.0	32.5	30.2	30.0	393.2
26	32.5	33.0	33.0	178.5	31.7	27.2	28.5	275.5**
Electrophoresis – Alpha 2 (%)								
0	14.00	15.75	15.75	13.00	13.25	14.50	16.25	13.50
4	13.75	15.75	15.25	13.50	14.50	13.00	16.75	10.50**
13	9.50	10.00	10.25	9.00	9.75	8.75	7.75	6.50**
26	9.25	9.75	9.75	9.25	10.75	9.00	8.25	7.25**

***p<0.050; n=4/sex*

Ophthalmology findings: Ocular findings were considered incidental, occurring in controls at similar incidence rates, and were not treatment-related.

Electrocardiology findings: There were no treatment-related changes in the heart rates of dosed animals when compared to control rates. Electrocardiograms were obtained from all animals at week 25. Whilst no distinct treatment-related changes were noted, all of the deflections in the 4500 ppm dose group including the P-wave, the QRS complex and the T-wave tended to be lower in most of the tracings when compared to

controls. The significance of the finding is unknown. There may be a relationship to decreased absolute heart weights in the same animals.

Pathology: A decrease in mean terminal body weight of both sexes in the 4500 ppm dose group (15% males and 26% females) was observed compared to controls (Table 9). Increases in the mean relative liver weight (26% males and 13% females) in the 4500 ppm group and in the mean absolute (39% males and 19% females) and relative (61% males and 75% females) kidney weights in the 4500 ppm group was also observed. These changes were considered to be treatment-related due to associated changes noted in the histopathological examination of the 4500 ppm dose group.

Mean absolute and relative pituitary weights in males at doses of 1500 and 4500 ppm, and of the spleen and adrenals of males at 4500 ppm were higher than control values. In the absence of any histopathological abnormalities in these tissues and only marginal differences, none of these findings were considered treatment-related. There was also a dose-related decrease in the mean absolute and relative prostate weights in males but no associated histopathological alterations. The study authors noted that considerable variation in prostate weights of adult control beagles had been observed in the past. Therefore, the significance of this finding is unknown. Various other changes in absolute organ weights at 4500 ppm were considered related to body weight decreases.

Table 9: Mean absolute and relative organ weights

	DIETARY LEVEL (PPM)							
ORGAN	0		500		1500		4500	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
Absolute (grams)								
Terminal body weight (kg)	10.98	10.53	10.10	11.38	11.93	10.33	9.35	7.78
Liver	222.00	226.25	226.75	216.75	278.25	228.00	238.75	174.00*
Kidneys	47.02	43.88	49.63	50.43	57.68	45.63	65.40	52.28
Heart	92.20	86.43	89.73	86.38	88.08	82.60	75.95	60.73*
Pituitary	0.064	0.081	0.060	0.079	0.075	0.075	0.076	0.064*
Spleen	24.33	24.50	23.63	27.43	26.65	25.00	28.33	20.90
Adrenals	1.03	1.22	1.03	1.34	1.09	1.20	1.13	1.11
Prostate	9.02	-	6.78	-	5.98	-	5.02	-
Relative (expressed as % ratio to terminal bodyweight)								
Liver	2.02	2.16	2.26	1.92	2.40	2.21	2.55	2.43
Kidneys	0.44	0.42	0.50	0.45	0.50	0.44	0.70	0.74
Heart	0.86	0.82	0.90	0.77	0.75	0.80	0.81	0.83
Pituitary	0.0006	0.0008	0.0006	0.0007	0.0007	0.0008	0.0008	0.0009

ORGAN	DIETARY LEVEL (PPM)							
	0		500		1500		4500	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
Spleen	0.22	0.23	0.24	0.25	0.23	0.24	0.30	0.27
Adrenals	0.010	0.012	0.010	0.012	0.009	0.012	0.012	0.016
Prostate	0.084	-	0.068	-	0.048	-	0.053	-

** $p<0.05$; n=4/sex*

Histopathology: Administration of 1500 and 4500 ppm resulted in histopathological changes in the livers and kidneys of both sexes. The lesions consisted primarily of bile stasis, focal hepatocellular degeneration and necrosis, and focal proximal tubular nephrosis. Bile stasis, both canalicular and cholangiolar, was noted in 1/4 females at 1500 ppm, 3/4 males at 4500 ppm and 3/4 females at 4500 ppm. Varying degrees and forms of hepatocellular degeneration were observed. Changes most prominent in the centrilobular region included cellular swelling with condensation of intracytoplasmic organelles into eosinophilic hyaline-like droplets and the plasma membranes were occasionally disrupted. Additional degenerative changes included a ballooning degeneration, lacking acidophilic droplets and a diffuse vacuolar degeneration in one male at 1500 ppm. Sections of liver in one male at 4500 ppm contained areas of sinusoidal ectasia with subsequent atrophy of adjacent hepatocytes and focal haemorrhage with a mild nonsuppurative inflammatory infiltrate in the portal areas. Lesions in the kidney noted in 2/4 females at 1500 ppm, 3/4 females at 4500 ppm and 2/4 males at 4500 ppm were very mild, focal and involved primarily the proximal convoluted tubules. The lesions were epithelial degeneration or nephrosis.

Conclusions:

The NOEL in dogs following 26 weeks of dietary exposure was 500 ppm of the 20% aqueous solution (that is, 100 ppm of polihexanide or approximately 2.5 mg/kg bw/d), based on histopathological alterations in the liver and kidneys (bile stasis, focal hepatocellular degeneration and necrosis, and focal proximal tubular nephrosis) at higher doses.

4 CHRONIC TOXICITY STUDIES

4.1.1 Dogs

Horner SA (1995) Polyhexamethylene Biguanide: One-year Dietary Toxicity Study in Dogs. Study performed by Zeneca Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK. Report No. CTL/P/4488; Study No. PD0947. Report date: March 1, 1995. Unpublished. Study period: May 1993 – May 1994.

GLP and QA: Yes.
Guidelines: No.

Materials and Methods

Polihexanide (batch reference number D4097, CTL reference number Y00156/008; Vantocil P, a 20.2% w/w solution in water supplied by Zeneca Specialities) was mixed in the diet and fed to 4 Beagle dogs /sex/dose for 1 year at levels of 0, 300, 1500 or 3000 ppm (equivalent to 0, 9, 46 and 91 mg/kg bw/d for males and 0, 9, 45 and 91 mg/kg bw/d for females). The original high dose level of 4500 ppm was reduced to 3000 ppm from day 1 of week 11 or 12 of treatment, due to unexpected mortalities and clinical signs. There was no rationale given for the choice of these dietary levels. Dogs were sourced from Zeneca Pharmaceuticals, Alderly Park, Macclesfield, Cheshire, UK and housed individually under standard conditions. Dogs were approximately 17–20 weeks old at the start of the experiment. Male dogs received 400 g and females received 350 g of laboratory diet (± test substance) each morning. Water was available *ad libitum*. Dogs were observed daily for mortalities or clinical signs. All dogs were given a full clinical examination pre-study, during weeks 13, 26, 39 and prior to termination which included cardiac and pulmonary auscultation and indirect ophthalmoscopy. Body weight was recorded on day 1 of study and then at weekly intervals. Any uneaten food was weighed on a daily basis throughout the treatment period.

Blood samples were taken from all dogs prior to feeding at weeks -1, 4, 13, 26 and 52 (prior to termination). The following haematology parameters were analysed: haemoglobin, haematocrit, red cell count, mean cell volume, mean cell haemoglobin, total and differential white cell count and platelet count. The following clinical chemistry parameters were analysed: alanine transaminase, aspartate transaminase, creatine kinase, alkaline phosphatase and gamma-glutamyl transferase activity, urea, creatinine, glucose, albumin, total protein, triglycerides, cholesterol, total bilirubin, calcium and phosphorous (as phosphate), sodium, potassium and chloride. Urine was collected pre-experimentally, in weeks 26 and 52 and analysed for the following: volume, pH, specific gravity, protein, glucose, bilirubin, urobilinogen, ketones and blood. All survivors were sacrificed at the end of the 1-year treatment period by exsanguination under sodium pentobarbitone anaesthesia. These, along with any dogs that died during the study, were subjected to a full necropsy. The following organs were weighed: adrenal glands, brain, epididymides, kidneys, liver, testes and thyroid gland. The following organs were histopathologically examined: adrenal gland, aorta (abdominal), bone (femur+stifle joint), bone marrow (sternum), brain, caecum, cervix, colon, duodenum, epididymis, eye, gall bladder, heart, ileum, jejunum, kidney, liver, lung, lymph node (mesenteric), lymph node (prescapular), mammary gland, prostate, rectum, salivary gland (submandibular), sciatic nerve, skin, spinal cord, spleen, stomach testis, thymus, thyroid gland, parathyroid gland, trachea, urinary bladder, uterus, voluntary muscle (semimembranosus) and grossly abnormal tissues. The statistical methods used for analysis of test parameters were considered appropriate. The following parameters were analysed: bodyweight, haematology, clinical chemistry, urinalysis and organ weight.

Results

Dietary analysis of polihexanide: Measurement of achieved concentration, homogeneity and stability in diet was not performed due to the lack of a reliable method for quantitative analysis for polihexanide in the diet and problems encountered in extracting the compound from the diet. However, nominal concentrations were analysed for each large batch of diet prepared at 300 and 4500 ppm (approximately 60 kg batches) with percentage deviations of within 6–15% of the overall mean.

Mortalities and clinical signs: Three of 4 males and one of 4 females receiving 4500 ppm/3000 ppm were killed prematurely during the study (as early as week 9 for males and week 35 for the female) after displaying severe and unexpected clinical signs of toxicity. This included marked reddening/peeling of scrotal skin (noted as early as week 3), lack of appetite and body weight loss (noted as early as week 5) and/or indications of hepatotoxicity (elevated plasma ALT and AST as early as weeks 4 and 8). The original high dose level of 4500 ppm was reduced to 3000 ppm from day 1 of week 11 or 12 of treatment due to these findings. Similar scrotal lesions were also apparent for the surviving male dog at 4500 ppm. Lack of appetite and body weight loss was also apparent for two of the three surviving females at this dose. Scrotal skin reddening, peeling, scabbing and/or dryness, with flaky scaly appearance was observed in males at the same incidence (2/4 animals) in control, 300 ppm and 1500 ppm groups, but not as marked as those seen for the 4500 ppm/3000 ppm group. Various other clinical findings were not considered treatment-related as they were either sporadic in nature or occurred at similar incidences as in controls. Full clinical examination of all dogs revealed no treatment-related ophthalmoscopic abnormalities or effects on pulse rate or body temperature.

Body weight and food consumption: A significant loss of body weight was observed in both sexes receiving 4500 ppm. Following reductions of the high dose level to 3000 ppm from weeks 11/12, no significant effects on bodyweight were observed compared to controls. A corresponding pattern for food consumption was observed, in that a significant reduction was noted in both sexes at 4500 ppm but was comparable to controls upon dose reduction to 3000 ppm.

Haematology, clinical chemistry and urinalysis: There were no treatment-related effects on any of the haematology parameters tested. Marked increases in plasma ALT and AST were noted in animals that were prematurely sacrificed. A marked increase in plasma ALT (by 90%) was observed in a few surviving animals at a dose of 4500 ppm/3000 ppm from as early as week 8. Due to the variability within the group (large standard deviations), the higher mean values only achieved statistically significant differences from controls at weeks 13/42 in males and weeks 13/17/21/26 in females. Decreases in plasma cholesterol (by 25%) in surviving animals were considered treatment-related at the high dose of 4500 ppm/3000 ppm (Table 10). As shown, cholesterol values in female dogs were statistically significantly reduced from weeks 4–52 (22–47%) in comparison with both pre-experimental values and/or concurrent study controls.

Table 10: Treatment-related mean clinical chemistry alterations in dogs[1]

	DIETARY LEVEL (PPM) [2]							
WEEK	MALES				FEMALES			
	0	300	1500	4500/3000	0	300	1500	4500/3000
Plasma cholesterol (mg/100 mL)								
Week 0	121	125	135	120	153	145	135	162

WEEK	DIETARY LEVEL (PPM) [2] MALES				FEMALES			
	0	300	1500	4500/3000	0	300	1500	4500/3000
Week 4	156	153	145	124**	174	175	148*	136**
Week 13	153	138	140	117*	181	183	162	111**
Week 26	150	138	123	113	191	179	153*	91**
Week 52	133	130	122	132	170	179	180	102**
Plasma Aspartate Transaminase (IU/l)								
Week 0	17.5	16.8	19.0	18.5	17.5	16.5	17.0	15.8
Week 4	14.8	17.9	14.7	29.6*	21.1	16.2	16.2	25.8
Week 10	22.2	26.9	15.4	28.0	16.1	15.1	13.1	67.1
Week 13	17.2	16.8	19.2	24.4	15.9	17.8	24.8*	19.5
Week 26	20.9	17.6	19.8	27.8	14.0	17.9	23.2	22.3
Week 42	24.8	16.1	23.1	28.2	21.1	20.5	18.1	20.5
Week 52	20.3	15.2	18.8	25.0	16.7	24.4	54.6	13.8
Plasma Alanine Transaminase (IU/l)								
Week 0	19.8	16.0	21.5	19.5	18.0	18.8	18.3	16.3
Week 4	19.6	22.1	25.2	29.4	25.1	19.0	24.8	20.3
Week 8	25.4	34.5	25.2	232.5	20.8	22.5	29.4	89.1
Week 10	22.5	22.5	25.0	70.5	2.6	117.2	81.8	198.9
Week 13	25.7	30.5	35.5	53.7**	25.0	24.4	35.6	53.9**
Week 17	31.3	33.9	35.8	57.0	23.4	29.3r	40.4	64.9**
Week 21	30.0	28.7	32.3	61.3	22.0	36.1	44.5	60.2*
Week 26	33.3	30.4	39.0	88.5	27.2	31.3	47.3	103.7*
Week 42	39.8	34.8	40.5	145.4**	25.2	28.3	35.1	43.0
Week 52	40.4	35.1	34.2	67.5	30.1	44.3	74.2	47.9

*n=4/sex; [1] The 4500 ppm dose was reduced to a level of 3000 ppm on day 1 of weeks 11 or 12. *p<0.05, ** p<0.01*

Urinalysis test results did not reveal any treatment-related changes.

Pathology: Relative organ weights were not reported in this study. The absolute adrenal weights of male dogs were statistically significantly increased at a dose of 3000 ppm by 23% *versus* controls (Table 11). The absolute kidney weights of the surviving male dog were statistically significantly increased at a dose of

3000 ppm by 54% *versus* controls. In the absence of corresponding histopathological findings, the increases in adrenal and kidney weights were not considered treatment-related. The absolute liver weight of male dogs was decreased by 14% at a dose of 3000 ppm, but was not statistically significantly different from controls. The absolute weights of the testes were statistically significantly decreased at a dose of 3000 ppm by 53% *versus* controls. These effect were considered treatment-related due to the histopathological observations noted at this dose level.

Table 11: Mean absolute organ weight changes in dogs [1]

	MALES				FEMALES			
	0	300	1500	3000	0	300	1500	3000 PPM
Adrenal glands	1.30	1.41	1.50	1.83*	1.60	1.65	1.67	1.65
Kidneys	55.8	58.4	62.5	85.8*	53.9	55.4	57.6	60.5
Liver	401	390	388	343	332	382	388	352
Testes	21.6	22.3	23.8	10.1*	-	-	-	-

[1] *At 3000 ppm, n=1 male and n=3 females*

** p<0.05*

Histopathology: Treatment-related changes were only observed at the highest dose of 4500 ppm/3000 ppm in the skin, liver, kidney and testis of dogs:

Dermatitis, featuring necrosis, parakeratosis and microabscess formation, was observed on the limbs and/or scrotum of 2 males and on the limbs and chin of one female that were sacrificed prematurely at a dose level of 4500 ppm/3000 ppm. Changes of this type are not common spontaneous lesions and were readily distinguishable from the mild inflammatory changes (acanthosis, hyperkeratosis and mononuclear cell infiltration) which do occur spontaneously in control and treated dogs.

Liver changes were characterised by the presence of large eosinophilic intracytoplasmic inclusion bodies in centrilobular and midzonal hepatocytes, and to a lesser extent cellular swelling and single cell necrosis.

Minimally increased hyaline droplet formation was observed in the kidney of all males that were prematurely sacrificed but not in the surviving male.

Bilateral testicular tubular degeneration was observed in 2/4 males (one that had been prematurely sacrificed and one that survived to termination). This resulted in a low testis weight for the surviving animal and was accompanied by Leydig cell hyperplasia.

Conclusion:

The NOEL in dogs following dietary exposure to polihexanide for 1 year was 1500 ppm (45 mg/kg bw/d) based on clinical chemistry changes in females (increased plasma ALT and decreased plasma cholesterol), decreased liver and testis weights in males and accompanying histopathological alterations at the highest dose (3000 ppm).

5 CHRONIC TOXICITY STUDIES - CARCINOGENICITY STUDIES

5.1 Oral

5.1.1. Mice

Milburn GM (1996) Two-Year Oncogenic Study In Mice. Zeneca Central Toxicology Laboratory, Macclesfield, UK. Report No. CTL/P/4649. Report date: June 26, 1996. Unpublished.

GLP and QA: Yes.
Guidelines: Not stated.

Materials and Methods

A 20.2% aqueous solution of polihexanide (sample D4079, no source or compositional analysis supplied) was mixed in the diet and fed to 55 C57BL/10JfCD-1/Alpk mice/sex/dose at 0, 400, 1200 or 4000 ppm of polihexanide (equivalent to 0, 54.7, 167 and 715 mg/kg bw/d of polihexanide in males and 0, 69, 216.5 and 855.5.3 mg/kg bw/d for females) for two years. The study report stated that due to extreme difficulty in extracting the compound from the diet, measurements of achieved concentration, homogeneity and stability in the diet were not performed. However, an aqueous solution of methyl violet (an ionically similar compound), equivalent to the lowest and highest dose levels of the experiment diet, was used to test the homogeneity of the mixing procedure. This analysis was performed approximately every six months and revealed that deviations from the overall mean concentration were within 10% of the nominal concentration.

Mortality and clinical signs were observed daily. Body weight and food consumption were recorded weekly for the first 12 weeks and twice monthly thereafter. Blood was collected from 11 mice/sex/dose in weeks 52 and 79 for haematological analysis. All animals that died or were killed in extremis and those sacrificed on schedule were subjected to gross pathological examinations. Absolute and relative organ weights were measured and included: liver, adrenals, kidneys, spleen, brain and testes. The following were examined microscopically: liver, kidney, spleen, heart, lung, adrenals, pancreas, gonads, thymus, thyroid, pituitary, pancreas, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, oral cavity, salivary gland and mesenteric lymph nodes, gall bladder, trachea, pharynx, larynx, ovaries, uterus, vagina, cervix, bones, skeletal muscle, skin, spinal cord, brain (cerebrum, cerebellum and pons), aorta, testes, epididymides, prostate and seminal vesicles, mammary gland, cervix, anus and rector-anal junction.

Results

The survival rate was comparable among all male groups including the control group (Table 12). In females, the survival rate at 4000 ppm was 12% lower than control at study termination. Anal swelling and discharge were the main treatment-related clinical observations. At 4000 ppm, there were 16 males and 5 females with swollen anuses. One female at 1200 ppm had anal swelling.

Table 12: Survival rate (%)

WEEKS	MALE				FEMALE			
	0	400	1200	4000	0	400	1200	4000
1	100	100	100	100	98	98	98	95
13	100	98	100	93	98	98	98	93
52	100	96	100	93	98	96	98	89
68	98	96	96	87	98	96	96	82
80	89	85	89	75	85	96	89	71
92	65	73	82	64	75	84	82	45
104	47	56	71	49	55	69	65	33

At 4000 ppm, body weight gain was severely reduced in both sexes during treatment compared to control animals despite an increase in food consumption (around 10–20% higher). By study termination, body weight gain was reduced by almost 50% in males and around 30% in females (Table 13). Haematological findings were unremarkable. Reticulocyte counts were not measured.

Table 13: Changes in mean bodyweight gain (g) for both sexes.

GROUP	MALE				FEMALE			
	0	400	1200	4000	0	400	1200	4000
Weeks 0–25								
bw gain	**11.4**	**11.7**	**11.1**	**8.2****	**7.4**	**7.4**	**7.4**	**7.0****
Weeks 0–51								
bw gain	**14.5**	**14.9**	**13.7**	**9.6****	**10.4**	**10.7**	**10.1**	**8.3****
Weeks 0–81								
bw gain	**15.8**	**15.9**	**14.0**	**8.9****	**11.9**	**12.5**	**10.8**	**8.0****
Weeks 0–105								
bw gain	**12.8**	**13.6**	**12**	**6.8****	**10.9**	**10.7**	**10.8**	**7.5****

**: p<0.05; **: p<0.01*

Pathology: There were no observed differences in organ weights or organ-to-body weight ratios. Table 14, Table 15 and Table 16 show the total gross pathological findings, non-neoplastic and neoplastic lesions. Gross examinations revealed an increased incidence of prolapse and/or swollen anus in both sexes at 4000 ppm (Table 12). This correlated with a dose-related increased incidence of inflammation and squamous cell hyperplasia at 1200 and 4000 ppm and an increased incidence of squamous cell carcinoma in both sexes at 4000 ppm. Adenocarcinoma was also observed in 1/49 male at 4000 ppm. The author suggested that the occurrence of tumours at this site was a consequence of chronic irritation and subsequent inflammation at the recto-anal junction.

Table 14: Selected total gross pathological finings

SITE	MALE				FEMALE			
	0	400	1200	4000	0	400	1200	4000
Gall bladder								
Distended	6 (11)	3 (5)	4 (7)	27 (49)	5 (9)	1 (2)	6 (11)	26 (47)
Anus								
Prolapse	0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Swollen	0 (0)	0 (0)	0 (0)	10 (18)	0 (0)	0 (0)	0 (0)	4 (7)
Caecum								
Distended	0 (0)	0 (0)	0 (0)	5 (9)	0 (0)	0 (0)	2 (4)	7 (13)
Liver								
Mass	5 (9)	3 (5)	7 (13)	14 (23)	4 (7)	2 (4)	3 (5)	8 (15)
Testes								
Reduced	9 (16)	9 (16)	8 (15)	17 (31)				

n=55/sex

The main areas of concern are the liver and rectal-anal junction. In the liver, an increased incidence of masses in both sexes at 4000 ppm correlated with increased incidences of haemangiosarcoma in the liver (Table 14 and Table 15). Most of the haemangiosarcomas were in animals that died well before study termination (most died between weeks 39–70), the earliest being 39 and 42 weeks in males and females, respectively. The incidence of haemangiosarcoma in concurrent controls was within the range of historical controls for mice which was provided by the author (2–15% for males and 0–9% for females). The incidence of haemangiosarcoma at 1200 ppm in both sexes was higher than the concurrent controls but was within the historical control range, and thus was not considered to be treatment-related (Table 15).

Table 15: Incidence of neoplastic lesions in mice

SITE	MALE				FEMALE			
	0	400	1200	4000	0	400	1200	4000
Liver								
Haemangiosarcoma	4/55 (7)	2/55 (4)	7/55 (13)	20/55 (36)	0/55 (0)	0/55 (0)	4/55 (7)	13/55 (24)
Increased extramedullary haemopoiesis	4/55 (7)	8/55 (14)	4/55 (7)	12/55 (22)	3/55 (4)	6/55 (10)	5/55 (9)	10/55 (18)
Rectal-anal junction								
Squamous cell	0/45	0/45	0/45	5/49	0/45	0/45	0/47	8/39

SITE	MALE				FEMALE			
	0	400	1200	4000	0	400	1200	4000
carcinoma	(0)	(0)	(0)	(10)	(0)	(0)	(0)	(20)
Adenocarcinoma	0/45 (0)	0/45 (0)	0/45 (0)	1/49 (2)	0/45 (0)	0/45 (0)	0/45 (0)	0/39 (0)

Note: Values in brackets represent percentage

Non-neoplastic lesions in the liver such as hepatocyte hypertrophy, induction of DNA synthesis and increased pigmentation (lipofuscin and haemosiderin) were also observed in both sexes at 1200 and 4000 ppm (Table 16). However the data suggest that such effects were not associated with the development of haemangiosarcoma. Instead, comparison of data from intercurrent animals (that died before the end of the experiment) and individuals that survived to the end of the study (terminal) indicated that animals that developed haemangiosarcoma frequently died prematurely and presumably before liver abnormalities such as hepatocyte hypertrophy could develop.

Increased extramedullary haemopoiesis in the spleen and distended gall bladder associated with luminal dilatation and epithelial hyperplasia were found in both sexes at 4000 ppm. There was a correlation between increased extramedullary haemopoiesis in the spleen (Table 16) and haemangiosarcoma (Table 15). At 4000 ppm, moderate to marked increased extramedullary haemopoiesis in the spleen was noted in 14/20 males and in 10/13 females that had haemangiosarcoma. It is possible that this association could have been due to bleeding from these vascular tumours and extramedullary hemopoiesis as a response.

Table 16: Incidence of non-neoplastic lesions in mice

SITE	MALE				FEMALE			
	0	400	1200	4000	0	400	1200	4000
Gall bladder								
Luminal dilatation	9 (20)	3 (7)	6 (13)	23 (48)	1 (2)	2 (4)	5 (9)	18 (39)
Epithelial hyperplasia	0 (0)	1 (2)	2 (4)	12 (25)	0 (0)	0 (0)	3 (6)	6 (13)
Liver								
Hypertrophy	0 (0)	1 (2)	7 (13)	29 (53)	0 (0)	1 (2)	19 (35)	27 (49)
Increased ploidy	0 (0)	1 (2)	7 (13)	29 (53)	0 (0)	1 (2)	20 (36)	21 (38)
Hepatitis	4 (7)	3 (5)	5 (9)	15 (27)	8 (15)	8 (15)	13 (24)	15 (27)
Pigmentation	0 (0)	0 (0)	3 (5)	20 (36)	0 (0)	1 (2)	6 (11)	23 (42)
Rectal-anal junction								
Inflammation	1 (2)	1 (2)	20 (44)	40 (84)	10 (21)	4 (9)	22 (47)	29 (74)
Squamous epithelial hyperplasia	0 (0)	0 (0)	5 (11)	12 (24)	0 (0)	0 (0)	3 (6)	8 (21)
Squamous metaplasia rectal glands	0 (0)	0 (0)	1 (2)	7 (14)	0 (0)	0 (0)	1 (2)	2 (5)

SITE	MALE				FEMALE			
	0	400	1200	4000	0	400	1200	4000
Spleen								
Increased extramedullary haemopoiesis	9/55 (16)	7/55 (13)	5/55 (9)	19/55 (34)	17/55 (31)	14/55 (25)	14/55 (25)	24/55 (43)

Conclusion:

The NOEL was 400 ppm (equivalent to approximately 55 mg/kg bw/d) in this 2-year carcinogenicity study, based on toxicity in the liver and the lesions at the recto-anal junction at higher doses. An increased incidence of haemangiosarcoma was seen in the liver and of squamous cell carcinoma at the recto-anal junction at 4000 ppm, the highest dietary dose tested.

5.1.2. Rats

Horner SA (1996) Polyhexamethylene biguanide: Two-Year Oncogenic Study in Rats. Zeneca Central Toxicology Laboratory, Macclesfield, UK. Report No. CTL/P/4663. Report date: June 26, 1996. Unpublished.

GLP and QA: Yes.
Guidelines: Not stated.

Materials and Methods

A 20.2% aqueous solution of polihexanide (sample D4079, no source or compositional analysis supplied) was mixed in the diet and fed to 64 Wistar rats/sex/dose at 0, 200, 600 or 2000 ppm (equivalent to 0, 12.1, 36.3 and 126.1 mg/kg bw/d of polihexanide in males and 0, 14.9, 45.3 and 162.3 mg/kg bw/d for females) for two years. Twelve rats/sex/dose were designated for interim sacrifice at 52 weeks with the remaining animals continuing to terminal kills after 105 weeks. The study report stated that due to extreme difficulty in extracting the compound from the diet, measurements of achieved concentration, homogeneity and stability in the diet were not performed. However, an aqueous solution of methyl violet (an ionically similar compound), equivalent to the lowest and highest dose levels of the experiment diet, was used to test the homogeneity of the mixing procedure. This analysis was performed approximately every six months and revealed that deviations from the overall mean concentration were within 10% on the nominal concentration.

Mortality and clinical signs were observed daily. Bodyweights and food consumption were recorded weekly for the first 14 weeks and twice monthly thereafter. Blood was collected from 13 rats/sex/dose of the main group in weeks 14, 27, 53 and 79 for haematological and clinical chemistry examinations. At the interim kill in week 53 and at study termination in week 105, blood was collected from all surviving animals. All animals that died or were killed in extremis and those sacrificed on schedule were subjected to gross pathological examinations. Absolute and relative organ weights were measured and included: brain, liver, heart, lung, adrenals, kidneys and spleen. The following were examined microscopically: liver, kidney, spleen, heart, lung, adrenals, pancreas, gonads, thymus, thyroid, pancreas, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, salivary gland and mesenteric lymph nodes, gall bladder, trachea, pharynx, larynx, ovaries, uterus, vagina, cervix, bones, skeletal muscle, skin, spinal cord, brain (cerebrum, cerebellum and pons), aorta, testes, epididymides, prostate and seminal vesicles.

Results

The survival rate was comparable among all male groups including the control group (Table 17). In females, the survival rate was 13% lower in the 2000 ppm group than in controls. There were no overt signs of toxicity or abnormal behaviour observed during the study.

Table 17: Survival (%)

WEEKS	MALES				FEMALES			
	0	200	600	2000	0	200	600	2000
1	100	100	100	100	100	100	100	100
13	100	100	100	100	100	100	100	100
52	100	97	97	92	100	100	98	82
68	94	93	89	88	96	94	91	88
80	85	83	78	81	88	88	81	80
92	79	63	58	68	83	85	79	71
104	42	40	41	45	52	65	56	39

Female rats fed 2000 ppm polihexanide had a decrease in bodyweight gain compared with control animals (10–15%; $p<0.05$). Food consumption and food efficiency, however, was not affected by treatment (Table 18).

Table 18: Changes in mean bodyweight gain (g) for both sexes.

GROUP	MALE				FEMALE			
	0	200	600	2000	0	200	600	2000
Weeks 0–-26								
bw gain	**375**	**378**	**367**	**350**	**156**	**150**	**153**	**143**
Weeks 0–52								
bw gain	**457**	**460**	**448**	**432**	**206**	**195**	**191**	**180***
Weeks 0–80								
bw gain	**451**	**457**	**452**	**432**	**260**	**255**	**245**	**222***
Weeks 0–104								
bw gain	**329**	**359**	**363**	**338**	**233**	**239**	**225**	**203***

**-Statistically significant, p<0.05*

Haematology, clinical chemistry and urinalysis: There were no statistically significant differences in haematological and urinalysis values when compared to their respective control values. Plasma ALP activity was increased in female rats at 2000 ppm during the study (Table 19).

Table 19: Plasma ALP activity

WEEKS	MALES				FEMALES			
	0	200	600	2000	0	200	600	2000
14	179	173	176	244**	117	111	120	167**
27	176	168	165	202	90	86	99	143**
53	206	178	179	213	79	78	75	122**
79	166	143	148	166	72	68	67	106**
105	142	159	145	154	74	79	65	129**

***p<0.01; n=13/sex*

Pathology: There were no observed differences in organ weights or organ-to-body weight ratios. Gross examinations of organs revealed no remarkable findings. Histologically, haemangioma in the liver was observed in 2/64 males and 2/64 females at 2000 ppm. Haemangiosarcoma in the liver was observed in 1/64 females at 2000 ppm (Table 20). With one exception, all of the vascular neoplasms in the liver were observed only at the terminal necropsy. One female with hepatic haemangioma was killed in week 91 in a moribund condition for reasons unrelated to the vascular neoplasm. The author stated that in 18 carcinogenicity studies in Zeneca laboratories (number of animals unspecified) using this strain of Wistar rat, there had been one liver haemangiosarcoma and no haemangioma in males. No findings of haemangiosarcoma or haemangioma in the liver had been observed in female rats. Since these tumours in the liver are rare in this strain of rats and similar lesions were observed in the mouse carcinogenicity study, they were considered to be possibly treatment-related. However Busey (1996) reported on a “pathology working group peer review” which concluded that the increased incidence of vascular neoplasms in the high dose animals was not treatment-related but represented incidental, sporadic cases.

Table 20: Incidence of liver haemangioma and haemangiosarcoma in the liver

WEEKS	MALES				FEMALES			
	0	200	600	2000	0	200	600	2000
Haemangioma	0	0	0	2	0	0	0	2
Haemangiosarcoma	0	0	0	0	0	0	0	1

n=64/sex

Conclusion:

The NOEL was 600 ppm (equivalent to approximately 36 mg/kg bw/d of polihexanide) in this 2-year feeding study, based on decreased bodyweight gain, increased ALP activity and the presence of haemangioma and haemangiosarcoma in the liver at the highest dietary dose tested of 2000 ppm.

5.2 Dermal

5.2.1. Mice

Clapp MJL (1990) Eighty-Week Skin Painting Study in Mice. ICI Central Toxicology Laboratory, Macclesfield, UK. Report No. CTL/P/331. Report date: July 1990. Unpublished.

GLP and QA: No.
Guidelines: Not stated.

[This study is a 1990 reformat of a study originally conducted in 1977.]

Materials and Methods

An aqueous solution containing 20% polihexanide (batch number SDC/596; 20% w/v purity; sourced from Imperial Chemical Industries Limited), was applied to the clipped dorsal skin of 50 mice/sex/dose; 4–5 weeks old; specific pathogen free colony maintained at Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderly Park, Macclesfield, Cheshire, UK. Mice were given dermal doses of 0, 0.6, 6.0 or 30 mg of polihexanide for five days per week over an 80-week period.

Mice were observed daily for mortality, clinical signs and any other abnormalities. Body weight and food consumption were recorded weekly for the first 12 weeks and twice monthly thereafter. All animals that died or were killed in extremis and those sacrificed on schedule were subjected to gross pathological examinations. The following were examined microscopically: liver, kidney, spleen, heart, lung, adrenals, pancreas, gonads, thymus, thyroid, pituitary, pancreas, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, oral cavity, salivary gland and mesenteric lymph nodes, gall bladder, trachea, uterus, skin, testis and prostate.

Mortality: Survival rates in both sexes at 30 mg/mouse were slightly lower than in other groups during the first year. The pattern continued throughout the remainder of the study resulting in low survival rates in this group at study termination (Table 21).

Table 21: Survival (%)

WEEKS	MALES				FEMALES			
	0	0.6	6.0	30	0	0.6	6.0	30
52	88	98	92	78	90	96	86	82
68	78	92	84	62	78	80	72	64
80	68	72	74	22	72	68	64	22

Clinical signs: Clinical signs were restricted to the highest dose. Irritation to the skin was noted in both sexes immediately after application. Erythema was noticed during the first few weeks and after the 4th week hyperkeratosis became evident, especially in males. The incidence of hyperkeratosis and desquamation progressively increased until 6–9 months and then remained at this level for the remainder of the study at which time 65% of males had hyperkeratosis and 49% had desquamation, while 23% of females had hyperkeratosis and 28% had desquamation compared to 0% and 2.7%, respectively for controls. Bilateral

protrusion of the eyes (exophthalmos) was noted in some animals at week 22 but gradually increased to about 13% for males and 50% for females by week 80. The incidence rapidly increased to greater than 90% in both sexes by week 44 and remained at this level until the study termination. Clinical examination of the eyes revealed no lesions in the cornea or retina in the early stages of the study. Subsequent examination of the eyes at weeks 64 and 73 revealed a similar pattern, although keratitis was seen in many of the affected animals.

Body weight gain in the highest dose group was reduced during the study compared to control animals (by 50% for males and 17% for females; $p<0.01$). Food consumption and food efficiency, however, were not affected by treatment.

Pathology: Pathological changes were restricted to the highest dose and mainly in the liver. Variable degrees of inflammation were observed in both sexes. These ranged from small isolated pockets of inflammatory foci in portal tracts, accompanied by simple fatty changes to a more generalised severe form of hepatitis characterised by large scale exudation and massive necrosis. These hepatic changes may have contributed to the large number of deaths in this group during weeks 52–79. Haemangiosarcoma in the liver was observed in 2/50 (4%) females at 30 mg/mouse (Table 22). This was considered to be incidental to treatment since the occurrence of haemangiosarcoma was within the range of available historical controls (2–15% for male and 0–9% for female; Milburn, 1996). Clinical and histological examination of the eyes and orbital contents did not show evidence of any pathological abnormality that could account for the bilateral protrusion of eyes. Furthermore, gross and microscopic appearance of thyroids was also normal.

Table 22: Selected incidence of liver haemangioma and haemangiosarcoma in the liver

WEEKS	MALES				FEMALES			
	0	0.6	6.0	30	0	0.6	6.0	30
haemangioma	1	0	0	2	0	0	0	1
haemangiosarcoma	1	0	1	1	0	0	0	2

n=50/sex

Conclusion:

Doses were given in terms of mg/mouse, which is non-standard, and only an approximate NOEL can be given (6.0 mg/mouse, approximately 300 mg/kg bw/d, noting that this dose was only applied 5 days per week). There was no evidence of carcinogenicity in this study.

6 GENOTOXICITY

Table 23and Table 24 summarise the findings of *in vitro* and *in vivo* genotoxicity studies.

Table 23: Summary of *in vitro* Genotoxicity Studies

ASSAY	BACTERIAL STRAIN OR CELL TYPE	CONC. OR DOSE	BATCH / PURITY	METAB. ACT.	RESULT	REFERENCE
Reverse mutation in bacteria	S. typhimurium TA1535 TA1537 TA1538 TA98 TA100	0.32, 1.6, 8.0, 40, 200 & 500 µg/plate, with and without activation	BX2125/ Purity not presented	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Callander (1989)
Mammalian Chromosome Aberration Test	Human lymphocytes	25, 100 & 250 µg/ml for donor 1 & 25, 100 & 187.5 µg/ml for donor 2, with activation 5, 25 & 50 µg/ml, without activation for both donors.	BX2125/ Purity not presented	+, -	-, -	Howard CA (1989)

Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation.

Table 24: Summary of *in vivo* Genotoxicity Studies

ASSAY	SPECIES (STRAIN)	DOSE, ADMINISTERED AS SINGLE ORAL GAVAGE	BATCH / PURITY	RESULT	REFERENCE
Micronucleus (bone marrow cells)	Mouse C57BL/6JfCD-1	250 & 400 mg/kg bw	BX2125 Ex, Purity not presented	-	Randall V and Beck SL (1989)
Unscheduled DNA synthesis (hepatocytes)	Rat (Alpk:APfSD)	375, 750 & 1500 mg/kg bw	BX2125Purity not presented	-	Trueman RW (1989)

Results are expressed as +, positive; -, negative; +/-, equivocal.

All studies were performed according to standard methodology. The data showed that polihexanide is not genotoxic.

7 INTERNATIONAL TOXICOLOGY ASSESSMENTS

The APVMA has been in direct communication with its counterpart regulators in the US EPA and the European Union (EU) in relation to polihexanide.

7.1 United States

In 2004–05, the US EPA performed a major hazard and risk assessment of polihexanide. Evidence of carcinogenicity in test animal species had initially warranted the review of products containing polihexanide. There are some 12 swimming pool products containing polihexanide in the US. According to the US EPA report, polihexanide can cause ocular irritation/corrosion, dermal irritation and sensitisation, systemic toxicity (including toxicity to the liver and male reproductive organs) and carcinogenicity in two species of animals (rats and mice) at very high exposure levels. Reportedly, vascular system tumours (haemangiomas, and haemangiosarcomas) occurred in response to both oral and dermal exposure. However the US EPA hazard report concluded that polihexanide is not genotoxic and gives NOELs for carcinogenicity and other effects.

The US EPA risk assessment discusses concerns in relation to potential acute and chronic toxicity including those from certain types of residential and OHS exposure and from possible dietary exposure from products containing polihexanide used on food preparation surfaces. It also discusses the carcinogenic potential of polihexanide when administered to test animals at very high exposure levels. In relation to cancer risks, the US EPA's Cancer Assessment Review Committee classified polihexanide into the category "Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential" by oral and dermal routes. Based on this, the Agency has determined that a quantification of human cancer risk is not required.

7.2 European Union

In 2007, the agency responsible for regulation of biocides in the EU advised that polihexanide was scheduled for review in the EU. In February 2010 France, the rapporteur member state, submitted a proposal for harmonised classification and labelling (CLH) report which considered all human health and environmental endpoints.

According to the CLH report in terms of carcinogenicity, polihexanide can increase the incidence of benign and malign vascular tumours in female rats by oral route and in male and female mice by oral and dermal route. The tumours are induced mainly in the liver, which is one of the target organ of polihexanide and the increase is clearly seen at doses above the maximum tolerated dose However, it is also observed more equivocally at doses below maximum tolerated dose (mouse oral study at mid-dose and rat oral study at high dose). These increases are not considered incidental when considering the clear induction of vascular tumours at higher doses and they are considered biologically significant and attributed to treatment.

The report concluded that polihexanide is however not genotoxic *in vitro* and *in vivo* and a classification as carcinogenic category 3 (agent is not classifiable as to its carcinogenicity to humans); R40 (limited evidence of carcinogenic effect) is warranted.

8 CONCLUSION

The evidence of liver tumours and squamous cell carcinoma at the recto-anal junction in rodents is of some interest from a human health point of view. However it should be noted that polihexanide is associated with cancer in rodents only at high doses which are unlikely to be encountered in occupational or public settings, it does not appear to be genotoxic, and clear NOELs were demonstrated in animal carcinogenicity studies. Therefore the OCSEH does not regard the observed tumours as a barrier to continuing registration of products containing polihexanide.

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