V. Safety Tests

1. Acute Toxicity

Oral, dermal, subcutaneous, and intravenous acute toxicity of hymexazol was tested on mice (dd-SLC), rats (SD-SLC) rabbits and chickens. These LD_{50} values are given in Table I.

Table I

Animal	(sex)	Route	LD_{50} mg/kg body weight
Rat	(M)	Oral	4678
Rat	(F)	Oral	3909
Rat*	(M)	Dermal	>10000
Rat*	(F)	Dermal	>10000
Rat	(M)	Subcutaneous	1924
Rat	(F)	Subcutaneous	1884
Rat	(M)	Intravenous	>1000
Rat	(F)	Intravenous	>1000
Mouse	(M)	Oral	2148
Mouse	(F)	Oral	1968
Mouse	(M)	Subcutaneous	1297
Mouse	(F)	Subcutaneous	1167
Mouse	(M)	Intravenous	445
Mouse	(F)	Intravenous	514
Rabbit	(M)	Dermal	>2000**
Rabbit	(F)	Dermal	>2000**
Chicken	(M)	Oral	>1000

* Wistar-Imamichi strain rats.
** Data by Indusrial BIO-TEST Laboratories Inc., Ill., U.S.A.

2. Subacute Toxicity

Five-weeks studies

The toxicity by forced oral administration of hymexazol suspended in 0.5% tragacanth solution for 5 weeks was studied in female and male rats at 5 dose levels: 2000, 1500, 750, 375 and 150 mg/kg. Each group consists of 10 male and 10 female rats, Following results were obtained.

1. In the group treated with 2000 mg/kg, sporadical deaths of animals. six males and 4 females, were observed throughout the experimental period.

In the groups treated with less than 1500 mg, none died.

2. Body weight tended to be inhibited in both males and females, relating with the increase of dose. In the group treated with 2000 mg/kg, the inhibition was especially pronounced. However, males treated with 150 mg/kg exhibited the increase of body weight than the controls.

3. A tendency of decrease in food ingestion was observed in both sexes of all groups, especially in the males treated with 2000 mg/kg.

4. In 1–2 animals treated with more than 1500 mg/kg, gas production and swelling of stomach and intestine were noted.

5. In the liver-body weight ratio, a tendency of increase was noted, especially in the males treated with 2000 mg/kg.

6. Mild swelling of liver cells was noted histologically in the animals treated with 1500 mg/kg (2 males and 2 females) and 7 animals treated with 2000 mg/kg (3 males and 4 females). No remarkable changes were noted in other organs examined.

7. Hematological and clinicochemical tests revealed no remarkable changes.

Based on these results, the maximum safe dose of this drug was supposed to be less than 750 mg/kg.

Three months studies

Subchronic toxicity of hymexazol for 3 months was examined in rats (SD) and mice (ICR) by Ueda and Nishimura, Tokyo Dental College.

Due to the extremely low toxicity of hymexazol the maximum concentration of hymexazol in the food was determined to 20000 ppm (or 2% w/w of hymexazol). The content was decreased stepwise by the factor of 1/2: 10000, 5000 and 2500 ppm (or 1, 0.5 and 0.25% w/w hymexazol). None of the animal died among all groups.

A tendency of inhibition on body weight increase was noted in male and female rats groups treated with 2% dose, after about 10 to 12 weeks. In mice, however, no such tendency was noted during the treatment for 3 months.

Kidney and liver weight and liver-body weight ratio were increased in male and female rats and mice treated with high concentrations of hymexazol as compared with the control group.

Adrenal weight in mice was definitely increased in groups treated with 1 and 2% hymexazol as compared with the controls. No such symptoms were observed in rats.

Table II

	Concentration of hymexazol in ~~drinking water~~ diet	Hymexazol intake (mg)/ Kg body weight/day*
Definitely Toxic dose	20,000 ppm	approximately 1,200
Toxic dose	10,000 〃	〃 600
No effect dose	5,000—2,500 〃	〃 300—150

*The figures are calculated based on the assumption that a rat of 300 g body weight eats 20 g of food a day.

No influences on hematological picture were observed by the administration of hymexazol for 3 months.

These results are summarized in Table II.

3. Mutagenicity[1)]

Hymexazol and seven known or potential chemical mutagens were applied to agar plate cultures of *Escherichia coli* (B/r Try$^-$). After incubation, the cultures were examined for an increase in reverse mutation rate.

Forward mutation was tested by measuring the resistance of *E. coli* (B strain) against T4 phage, and the tolerance of *Salmonella typhimurium* (LT-2 strain) against azetidine carboxylic acid.

Another evaluation was conducted with the somatic male Chinese hamster cells (DON) grown in tissue culture, and chromosomal aberration frequencies were measured.

Hymexazol gave all negative results throughout the experiments even at very high concentrations whereas other known mutagens gave clear positive results in most of the cases. These results are summarized in Table III.

Table III. Mutagenicity and chromosomal aberrations induced by test chemicals.

System / Chemical	*E. coli* B/r Try-	T4 phage resistance	*S. typhimurium* LT-2	Chromosome aberration	Mutagenicity
Hymexazol	−	−	−	−	−
Acridine orange	+	−	−	+	+
2-Aminopurine	+	±	+	±	+
Ethyl methane sulfonate	+	+	+	+	+
Maleic hydrazide	−	−	−	±	±
N-Methyl-*N*-nitro-*N*-nitrosoguanidine	+	+	+	+	+
4-Nitroquinoline-1-oxide	+	+	+	+	+
Triethylenephosphoramide	+	+	+	+	+

Although the physiological differences between bacteria and mammals may not make the direct extrapolation of these results to mammals possible, it is desirable that no mutagenicity was demonstrated in the various systems employed. Furthermore, the negative result with mammalian system in vitro, together with the evidence for the rapid elimination of orally administered hymexazol from the body of rats[2)] indicates the safety of the compound on a genetic hazard to mammals.

4. Toxicity to Aquatic Animals

Acute toxicity of hymexazol to various aquatic animals was examined by Yoshida and Nishiuchi[3)] after the method described in the Direction B No. 2735 (Ministry of Agriculture and Forestry, Japan, 1965).

As Table IV shows, hymexazol was found to be extremely low in its toxicity, and expected not to cause injuries to aquatic animals when used under ordinary conditions. Consequently, it has been classified by the Government into Class A as one of the safest pesticides on fishery damages.

Table IV. Toxicity of hymexazol and other chemicals to aquatic animals.

	TLm (ppm calculated on active ingredient)						
	carp	wakin goldfish	killifish	mudfish	tadpole (toad)	crawfish (adult)	water-flea (adult)
	48(hr)	48	48	48	48	72	3
hymexazol	>40	>40	>40	830(l)	1000(l)	>40	>40
DDT	0.25	0.068	0.012	0.24	31(e)	0.40	>40
parathion	4.5	1.7	2.9	1.4	7.2	0.082	0.005
Zineb	>40	>40	>40	620(w)	380(w)	>40	>40
Maneb	1.8(w)	2.0(w)	3.3(w)	73(w)	40(w)	>40	10~40
PCP-Na	0.12	0.12	0.082	0.12	0.25	28	3.6

l: liquid formulation, e: emulsifiable concentrate, w: wettable powder

References

1) T. Tokuyama, H. Saito, S. Sudo and K. Usuki: "Studies on Mutagenicity of Pesticides", Nomura Research Inst., 1972.
2) M. Ando, T. Nakamura and M. Nakagawa: Agr. Biol. Chem., in press; Presented in part at the Annual Meeting of Agr. Biol. Chem. Soc. Japan, April, 1972.
3) K. Yoshida and Y. Nishiuchi: Bull. Agr. Chem. Inspect. Stn., No. 12, 122 (1972).