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BRIEF NOTE

THE EFFECT OF ALPHA-CHLOROHYDRIN ON THE FERTILITY OF MALE RATS[1]

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Several male antifertility compounds have been evaluated for use in rodent control (Marsh and Howard 1975). Only Alpha-chlorohydrin (3-chloro-1,2-propanediol, also known as U-5897), which has been investigated both in the laboratory and under natural conditions as a male antifertility agent (Ericsson 1970, Andrews and Belknap 1975), appears to have practical application in rodent control. At lower doses, it produces "functional" interference in the spermatozoa, causing "reversible antifertility" (Coppola 1969, Ericsson and Baker 1970). Higher doses result in permanent sterility due to epididymal lesions, which block sperm transfer from the testis to the epididymis via the ductili efferentes. Fluid accumulation in the testis causes pressure degeneration of the germinal epithelium; the testis atrophies, and the seminiferous tubules become aspermatic (Ericsson and Connor 1969, Ericsson and Norland 1970, Cooper *et al* 1974).

The higher dose effect (permanent sterility) appears to be limited to Norway rats (*Rattus norvegicus*) and black rats (*R. rattus*) (Kennelly *et al* 1970), whereas reversible antifertility effects may be produced in a variety of other mammals (Ericsson 1970). At a still higher dose (LD_{50} = 152 mg/kg), the compound is toxic to Norway rats (Ericsson and Baker 1970). Our purpose was to investigate the feeding patterns of wild and domestic Norway rats when exposed to a 1% α-chlorohydrin bait and to determine toxicity and antifertility effects.

Sprague-Dawley and wild Norway rats, trapped from a local horse barn, were caged individually and maintained on a diet of Purina lab chow *ad libitum* for 15 days. A commercial concentrate containing 20% α-chlorohydrin (EPIBLOC) produced by GAMETRICS Ltd. was utilized. The bait was prepared by mixing one part of concentrate with 19 parts of EPA bait (65% ground corn, 25% rolled oats, 5% corn oil, and 5% powdered sugar) to give 1% α-chlorohydrin formulation. The test animals were pre-baited with ground Purina lab chow for 3 days, then given a 3-day choice test with the α-chlorohydrin bait and untreated EPA bait; positions of the food bowls were alternated each day. During the 7-day post-treatment period, animals were given ground lab chow. Doses were calculated from the total amount of treated bait consumed.

After the post-treatment period, the test males were co-habited with females, which were checked every 4 hr between 0800 and 2400 hr for evidence of mating (vaginal plug and/or spermatozoa in vagina). Antifertility efficacy was assessed in terms of implantation sites found in mated females necropsied on day 14 of gestation or by counting the number of young produced. The epididymis was examined for gross anatomical lesions and for the presence of motile spermatozoa. Separate testicular and epididymal tissues were fixed in Bouin's solution and prepared routinely with H & E stains for microscopic examination.

All treated males were sterile, as determined by microscopic examination of the

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TABLE 1

Fertility of male Norway rats (Rattus norvegicus) treated with α-chlorohydrin for 3 days. Treated males were co-habited with females, 2-week post test followed in 2-weeks by necropsy of both males and females.

Rat Strain	Rat No.	Mean Body Weight (g)		Mean Bait Consumption		Dose* mg/kg	Mean No. Uterine Implants**	No. Treated Males with Macroscopic Epididymal Lesion	No. of Treated Males with Motile Sperm
		Initial	Final	EPA+	α-ch++				
Sprague-Dawley	10	206.5	234.5	6.3	1.1	51.6	0.0	9/10	2/10
Control	10	199.1	250.0	22.9	—	—	13.7	10/10	10/10
Wild	10	259.9	213.0	6.7	1.2	46.9	0.0	5/10	4/10
Control	5	248.2	222.2	17.2	—	—	9.0	5/5	5/5

*Dose (mg/kg [a.i.]) was calculated using initial body weight.
**Only 5 of the treated males were co-habited with females; the remaining 5 were necropsied 2 weeks post-test.
+EPA=65% ground corn, 25% rolled oats, 5% corn oil, and 5% powdered sugar.
++α-ch=Alpha-chlorohydrin.

testis and epididymis and/or breeding experiments. The lowest dose producing an epididymal lesion was 71.4 mg/kg, and lesions were localized in the ductuli efferentes and proximal caput epididymis. The epididymis distal of the lesion was devoid of spermatozoa, in agreement with the findings of Ericsson and Connor (1969), who reported that the immediate consequence of the lesion is blockage sperm transport through the epididymis.

Ericsson and Connor (1969) suggested

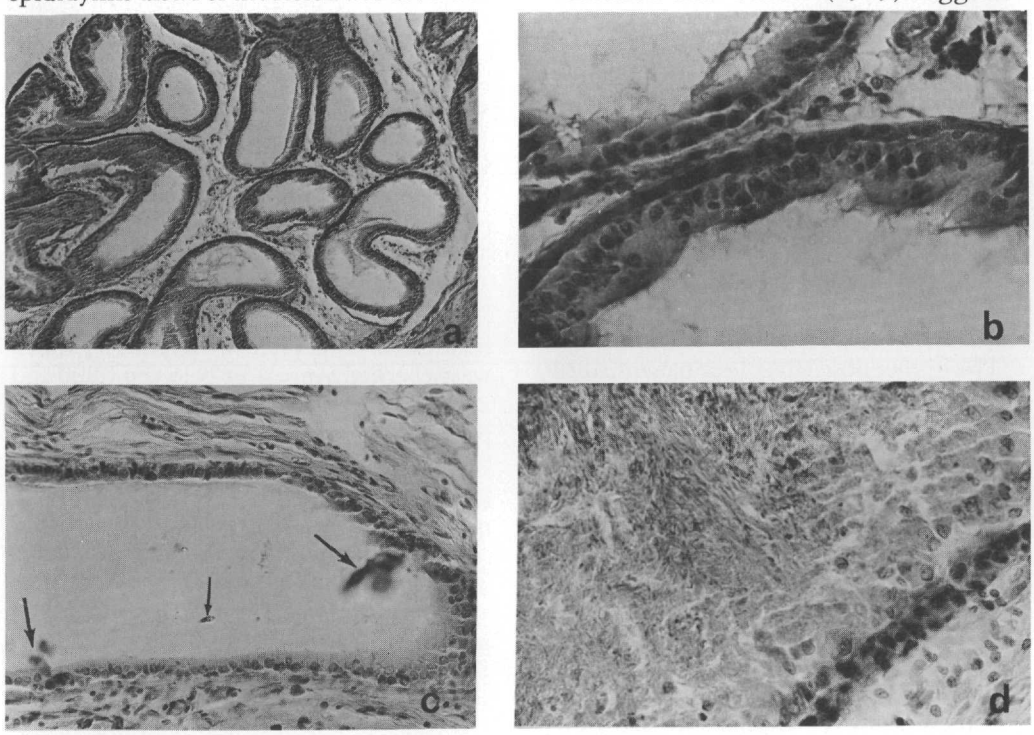

FIGURE 1. Initial segment of the caput epididymis five weeks after bait consumption (343.5 mg/kg): (1a) Desquamated epithelial cells and lumen devoid of spermatozoa x250; (1b) Characteristic edema of the epithelial cells x400; (1c) The lining epithelium has detached (arrows) and together with spermatozoa and macrophages will block the lumen (see 1d) x400; (1d) The lumen filled with sperm granuloma.

that a daily oral dose of 35 mg/kg for 7 days or a single oral dose of 45 mg/kg α-chlorohydrin was sufficient to produce an epididymal lesion. In our experiments, some rats consuming more than 45 mg/kg (but less than 85 mg/kg) did not have epididymal lesions but were unable to fertilize females (table 1). At higher doses ($\geq$ 100 mg/kg) macroscopic lesions with the formation of spermatocoel and sperm granuloma were observed. It is possible that a higher dose is required to produce lesions if the compound is administered with a bait rather than by oral intubation, as occurred in the former tests.

We found the epididymal lumen distal to the lesion devoid of spermatozoa (fig. 1a). In microscopic examination, inflammation of the epithelial tissue with noticeable edema (fig. 1b) and emigration of polynuclear leucocytes was evident (fig. 1c). The pseudo-stratified columnar cells had exfoliated, and the basal epithelial cells were separated from the basement membrane. The lumen of the proximal caput was filled with degenerating spermatozoa, desquamated epithelial cells, and polynuclear leucocytes (fig. 1d). The seminiferous tubules throughout the testis were shrunken and devoid of spermatozoa, but a few spermatogonial cells were retained (fig. 2). Ericsson (1970) reported that while most semniferous tubules of treated lab rats became inactive, a few sites of complete spermatogenesis were seen. We did not see this result.

Treated males induced vaginal plugs in females, indicating that α-chlorohydrin does not affect libido. This finding has practical implications, since sterilized but sexually active males can compete for mates and may induce pseudo-pregnancy, thus decreasing normal pregnancies (Marsh and Howard 1975). In a promiscuous species like the rat, however, this phenomenon is of decreased significance. While the use of the sterile male technique has been suggested for population management of rats (Glass 1974), the actual use of such a management tool has not been realilzed.

Only one of our treated males died, but

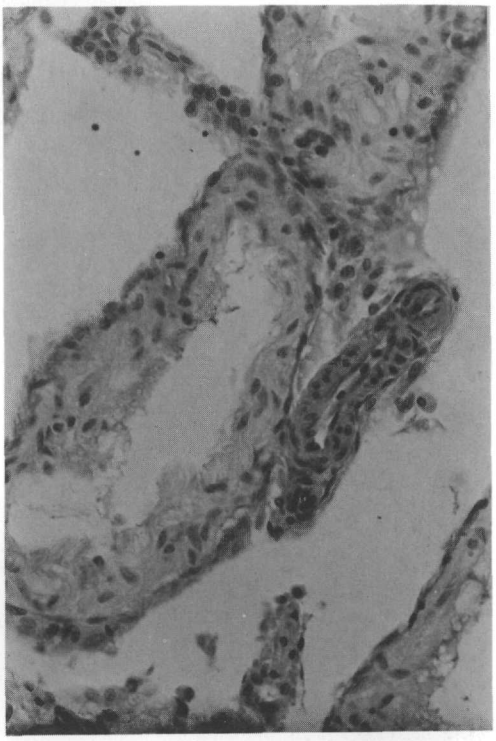

FIGURE. 2. Testis from treated rat showing (343.5 mg/kg); arrested spermatogenesis and degenerated germinal cells. A few germinal cells and Sertoli cells remain x250.

42% of the males consumed the equivalent of an acute lethal dose over 3 days (table 1). The first-day bait consumption was much lower, however, than the LD_{50} value (152 mg/kg) reported for this species (Ericsson and Baker 1970). According to Ericsson *et al* (1971), the drug has no cumulative toxicity and is rapidly detoxified and eliminated from the body through urine, feces, and breath in less than 24 hr (Jones 1975). Consequently, toxicity must be evaluated from a single-day dose.

In summary, α-chlorohydrin ingested with bait caused sterility in male rats by producing lesions in the epididymis and blocking sperm transfer via the dutuli efferentes; it inhibited spermatogenesis by destroying the germinal epithelial cells (70-100 mg/kg). Those animals of either sex receiving a greater dose ($\geq$ 200 mg/kg) were killed, but consumption in a single

day is required to cause mortality. Alpha-chlorohydrin could be a management tool for use against Norway rats, since males not killed are likely to be permanently sterilized but sexually active and compete for mates, thus reducing fertile matings.

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