Dept. of Genetics,
Fac. of Agriculture, Assiut Univ., Assiut, Egypt.
Head of Dept. Prof. Dr. H.I. Abdallah.

CHROMOSOMAL ABERRATIONS INDUCED BY AQUATIC WEED KILLER "REGLON" IN OREOCHROMIS NILOTICUS

(With One Table & 2 Fig.)

By

N.T. HAMDOON and A.SH. SEDDEK*

(Received at 11/12/1994)

التغيرات الكروموسوميه المستحدثه بواسطة مبيد الحشائش / الرجلون/ على أسماك البلطى النيلى

نصر الدين حمدون ، عبد اللطيف صديق

نظرا لاستخدام الرجلون كمبيد عشبى لمقاومة الحشائش المائيه فى الترع والأنهار دون أن تدرس خطورته على الأسماك التى تعيش فى مياهها . مما دفعنا لدراسة مدى خطورة هذه المبيدات على أسماك البلطى النيلى والتى تعد واحدة من أهم الأسماك النيليه فى مصر . وقد استهدف البحث دراسة التغيرات الكروموسوميه الناجمه عن استخدام هذا المبيد على البيئه المائيه (أسماك البلطى النيلى) .

وقد استخدم فى هذا البحث عدد ٧٥ سمكه تراوحت أوزانها بين ١٥ ، ٢٠ جرام عوملت لمدة ٩٦ ساعه . وقد تم استخدام نسيج الأمعاء فى اعداد التحضيرات الكروموسوميه التى تم فحصها .

وقد أظهرت نتائج هذا البحث ارتفاع معدل التشوهات الكروموسوميه بصفه عامه وان بدت فى أوجها فى الفجوات والكسور الكروموسوميه كما ظهرت تغيرات أخرى كالانتقالات الكروموسوميه وثنائية السنترومبير كما لوحظ وجود تكرار منخفض لبعض التغيرات الكروموسوميه الأخرى مثل زيادة أعداد الكروموسومات عن العدد الأصلى .

يتضح مما سبق الدور الخطير الذى يلعبه الرجلون المستعمل كمبيد عشبى فى احداث تشوهات كروموسوميه فى السمك مما يشير الى خطورته على الجهاز الوراثى للأحياء المائيه التى تعيش فى المياه الملوثه بهذا المبيد – وكذا على مستعملى هذه المياه من انسان وحيوان .

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*: Dept. of Forensic Medicine & Toxicology, Fac. of Vet. Med., Assiut Univ.

SUMMARY

The effect of aquatic weed killer Reglone on *O.miloticus* mitotic chromosomes was studied. Animals were exposed to water containing Reglone for 72 and 96 hours durations. Metaphases from intestine tissue were prepared, stained and scored for chromosomal aberrations. The results obtained showed that exposure of fish to water containing Reglone resulted in an enhancment in the frequency of metaphases exhibiting chromosomal aberrations (20.95% & 20.64% for 72 h. and 96 h., respectively virsus 6.5% for control fish). Single chromatid breaks and gaps were the highest aberrations compared to the gross chromosomal aberrations such as dicentrics and translocations. Other chromosomal abnormalities such as condensation, fragmentation and stickiness were observed in the treated aminals. The present results indicate the possbility of using chromosomal aberration analysis in *O.niloticus* as a useful tool for studying potentially dangerous waterborne chemicals *in vivo*.

Keywords: Chromosomal aberrations, aquatic weed killer, Reglon, Oreochromis niloticus.

INTRODUCTION

Studies on the effects of chemical mutagens on fish chromosomes are very limited. The majority of these studies have been carried out by Tsoi and his coworkers (*TSOI, 1970, TSOI, 1974 and TSOI* et al *., 1975*),during their investigations of chemically induced gynogenesis in *salmo irideus* and *coregonus peled*. Dimethul sulfate and nitrosomethyl urea induced chromatid and chromosome bridges in embryos developed from ferilization of normal eggs and treated sperms. Similar studies indicate the induction of chromosome abnormalities in the developing *gastrulas* of carp *(TSOI* et al*., 1975)*.

The modern cytogenetic techniques for chromosome analysis have been employed to investigate radiation and chemically induced damage to the genome of fishes *(KLIGERMAN, 1982)*. Mudmiunows exposed to trenimon, showed a dose-dependent increase in chromosome damage *(Sugatt, 1978)*. Genotoxic potentiality of the inorganic weedicide sodium arsenite in the experimentally treated Tilapia fish was investigated *(MANNA and MUKHERJEE, 1989* b*)*. They showed a relatively high frequency of metaphase chromosomal aberrations in the treated specimens as

compared to control. Some of these weed killer herbicides has a teratogenic effect in mice *(HOOD and BISHOP, 1972)*.

The cytogenetic effects of Reglon in fish have not been explored. Since weedicides in use are likely to contaminate water, the verification of its genotoxic potentiality is important using fish as a model. Therefore, the present study is carried out as an attempt to study the cytogenetic effects of the common used weedicide Reglon on Egyptian Nile fish *O. niloticus*.

MATERIALS AND METHODS

Seventy five *Oreochromis niloticus* Fishes weighting from 15-20 gm for each and of average length of 10 cm were divided into three groups. The first group was used as a control. The second and third groups were exposed to 18 ppm of Reglon (Tanlott's Hill, Research station bracknell, Berks, England) for 72 h. and 96 h. respectively.

Chromosome preparations were made by the solid tissue technique *(KLIGERMAN and BLOOD, 1977)*. Previously, fish were injected with 30 µl/g body weight of 0.05% colchicine. Five hours later, fish were sacrificed by decapitation and intestine tissue removed and treated with 0.4% Kcl hypotonic solution for 30 min. Tissues were then fixed in cold fresh 3:1 ethanol: acetic acid, which was changed after 1 hour. Cell suspension was made and placed on clean, heated slides. Slides were stained for 15 min.with 10% Giemsa, rinsed in distilled water and air dried. Metaphases were examined using 100X oil immersion lens and selected metaphases were photographed. Two tail t-test has been applied for testing the significance of the differnces between control and treated fish.

RESULTS

Table 1 shows the results obtained from cells analysed in the intestine tissues of both control and treated fish. Out of 492 metaphases analysed, 32 showed abnormalities (6.5%) and served as spontanous frequency of chromosomal aberrations in this investigation.

Treated fish for 72 hours showed that out of 358 metaphases, 75 cells (20.95%) exhibited chromosomal aberrations. Statistical analysis revealed a highly significant difference between control and 72 hrs. treated animals ($P<0.002$). Moreover, the scored metaphases from 96-hours treated fish showed that 155 cells from 751 (20.64%) exhibite chromosomal abnormalities. Again the t-test indicates a highly

significant difference between the frequency of aberrant chromosome cells of control and treated animals ($P<0.001$). In both treatments, the observed frequencies of chromosomal aberrations were almost about three times that of the control.

DISCUSSION

Regarding the types of the recorded abnormalities, both single chromatid gaps and breaks were the most dominant aberrations compared to gross chromosomal aberrations which were less frequent.

Figure (1) summarizes the obtained results which reflect the effects of treatment with such weed killer on *O.niloticus* fish genomes.

In addition to breaks and gaps, other abnormalities such as sticky chromosomes and hyperdiploidy were frequently observed in both treatments. Fig.2 (a, b & c) indicates some of aberrant metaphases observed in this investigation.

The results obtained are in agreement with the studies of Manna nd *MUKHERJEE, (1989 B.)* They found a higher frequency of chromosomal abnormalities in *O.mossambicus* treated with inorganic weedicide, sodium arsenite than that of untreated fish indicating its genotoxic effects. The same effect has been found in treating *O.mossambicus* with malathion and mercuric chlorid *(MANNA and MUKHERJEE, 1986, and 1989 A).* Genotoxic effects of Reglon found in the present investigstion are similar to studies of *AL-SABTI, (1985) and AL-SABTI* et al. *(1984)* on the effect of different kinds of water pollutant on rainbow trout.

The induction of chromosomal aberration in fish, as well as in other organisms, is a good parameter for estimating environmental hazards resulting from water contamination by pesticides and other agents. The present work indicated a mutagenic effect of the weedicide reglon on fish living in polluted water.

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Table (1): Types and frequency of chromatid and chromosome type abnormalities in control and Reglon treated fish.

Time post exposure	No. of examined metaphases	Metaphases with aberrations		single chromatid gaps		Single chromatid breaks		Iso-chromatid gabs		Iso-chromatid breaks		Dicentric chromosomes		Ring chromasomes		Translocations		Fragments		Other types	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	42	32	6.50	10	2.03	15	3.05	3	0.61	0.	0	0	0	2	0.41	0	0	0	0	0	0
72 hours	358	75	20.95**	15	4.19	26	7.26	6	1.67	1	0.03	5	1.40	1	0.03	4	1.11	1	0.03	18	5.03
96 hours	751	155	20.64**	50	6.66	50	6.66	9	1.2	2	0.27	6	0.80	3	0.04	9	1.20	4	0.53	32	4.26

** Highly Significant at ($P<0.01$).

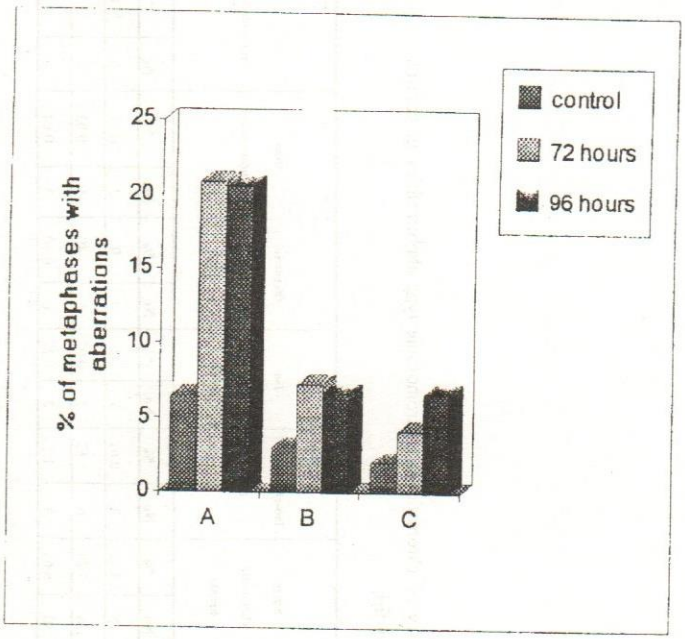

Fig. 1: Frequencies of aberrant metaphases obtained from control and Reglon treated fish.
A= total aberrations B= singel chromatid gaps C= singel chromatid breaks.

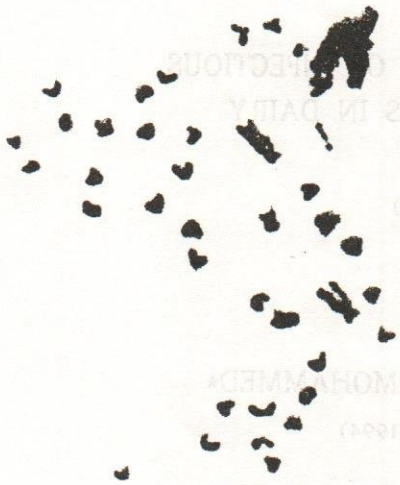

Fig. (2): Control

Fig. (2 a): Hyperdiploidy

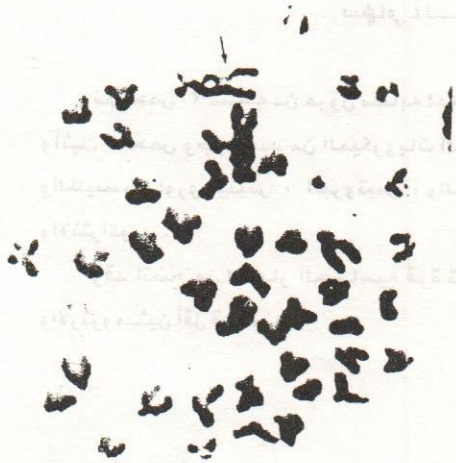

Fig. (2 b): Single chromatid break

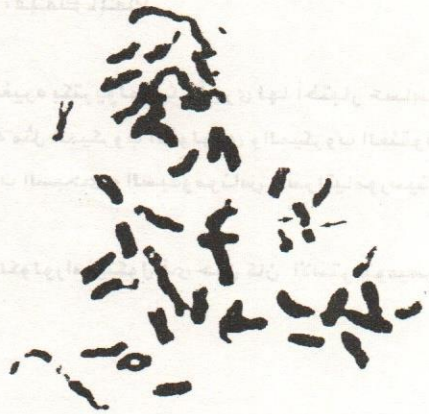

Fig. (2 c): Single and iso chromatid gaps