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PRELIMINARY NOTE ON THE APPEARANCE IN IVORY COAST OF RESISTANCE TO CHLORPHOXIM IN SIMULIUM SOUBRENSE/SANCTIPAULI LARVAE ALREADY RESISTANT TO TEMEPHOS (ABATE (R))

by

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Introduction

Resistance to temephos (OMS 786) in larvae of Simulium soubrense/sanctipauli (two members of the S. damnosum complex in West Africa) was confirmed at Chutes Gauthier on the Lower Bandama River in Ivory Coast in May 1980 (Guillet et al., 1980). The phenomenon has remained limited to S. soubrense/sanctipauli, but has spread to all of the river basins in Ivory Coast normally colonized by that species (Anon., 1981). Resistant S. soubrense larvae now comprise 100% of the S. damnosum s.l. population found in some savanna rivers treated with temephos. Before treatments, S. soubrense was present only as a small component of the S. damnosum s.l. population.

Chlorphoxim (OMS 1197), the only replacement insecticide then operational, was introduced provisionally while the testing of other larvicides continued. In 1980, chlorphoxim was first used at Chutes Gauthier for 11 weekly treatments, beginning in the week of 15 June. There was then a pause of seven weeks due to a lack of the chemical. Treatments were then resumed and continued up to the end of the year (10 more weeks). The results of this last series were considered satisfactory (1-2 flies/man/day), and treatments were suspended. They were not resumed until the week of 10 May 1981. During the suspension period, the monthly average of flies caught/man/day rose from 24 in January to 860 in May. Treatments continued from May until October 1981, but the number of flies fell very slowly, reaching two/man/day only in the first week of September. The catch began to rise again in the second week of September, and reached 2000/man/day in October at which time treatments were stopped and a programme of susceptibility testing began. This paper presents results of those tests.

Methods

Susceptibility tests were carried out with alcohol solutions of technical chlorphoxim (WHO sample) according to the method of Mouchet et al. (1977). This method was officially adopted by the WHO Expert Committee on Vector Biology and Control in 1981 (WHO, 1981) and has been used to gather almost all of the susceptibility data in the Onchocerciasis Control Programme Area. An important modification of the method reported here is the use of "old" or "mature" larvae (7th instar) in some tests. The official (Mouchet) test method requires the use of "young" (4th and 5th instar) larvae. The use of mature larvae was not recommended because the susceptibility of these larvae was found to be less than that of young larvae (Mouchet et al., 1977). Also, the mortalities with mature larvae were very heterogenous and log dose-probit mortality lines with narrow confidence intervals could not be prepared.

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However, there are several important advantages to the use of mature larvae. Under operational conditions it is often difficult to find large numbers of young larvae. In cases of treatment failure it is often the mature larvae which survive as subjects for susceptibility tests. Long treatment suspensions to allow young larvae to develop undisturbed are not always acceptable for epidemiological reasons. Mature larvae are more robust than young larvae and are easier to handle. Most important, mature larvae can be identified cytotaxonomically. This permits identification of larvae surviving diagnostic doses, which cannot be done with young larvae.

Because of these advantages, the question of using mature larvae was restudied, to determine if resistance could be detected by comparing "resistant" and "susceptible" mature larvae.

As it was believed that some of the heterogeneity of earlier results arose from the inclusion of 6th-instar larvae, the morphological criteria for selection were carefully reviewed. Mature larvae were then defined as those having the filaments of the pupal gills well developed and easily visible in the histoblasts. The histoblasts may be pale or dark. These criteria select mostly 7th-instar larvae (Grenier & Feraud, 1960; Kurtak, 1980).

Parallel tests were done with young and mature larvae from the same breeding site to establish a diagnostic dose for mature larvae. The morphological criteria were checked by examining groups of dead and surviving mature larvae. When it was initially found that among the dead larvae, there were consistently more smaller larvae with less-developed histoblasts than in the surviving group, the criteria were revised to exclude these younger larvae. Once the criteria were fixed as described in the preceding paragraph, the log dose-probit mortality lines obtained paralleled quite closely those for young larvae and had about the same heterogeneity. The mature larvae were found to be about 8-10 times less susceptible than young larvae, but this difference is constant for a given insecticide.

The establishment of diagnostic concentrations for mature larvae has greatly facilitated surveys for resistance.

It is important to exclude 6th-instar larvae from test samples, since it seems that it is during this instar that susceptibility changes rapidly.

Samples of mature larvae were preserved in Carnoy's fixative at the time of each test, for confirmation of cytospecies by chromosome staining. In tests with young larvae, a sample of mature larvae was taken from the same breeding site at the same time. In tests with mature larvae, surviving test larvae themselves were preserved.

When "diagnostic concentration" is referred to, it is equal to 0.0125 mg/l of chlorphoxim for young larvae and 0.125 mg/l for mature larvae.

Probit analysis of the data was carried out by the method of Maximum Likelihood (Finney, 1971) using a Texas Instruments TI 59 Calculator and a programme developed by Dr B. Grab of the Health Statistical Methods Unit, WHO, Geneva.

Results

The results of susceptibility tests at Chutes Gauthier and other sites in the Bandama Basin are presented in Table 1 and Figs 1 and 2. Data from sites outside the Bandama Basin are given in Table 2. A map, Fig. 4, gives locations of the test sites. Comparative data for normal mature larvae from other sites are presented in Table 3 and Fig. 3 (no such data exist for Chutes Gauthier). Due to the large number of lines in Figs 1 and 2, it is not possible to display individual data points. However, statistical analysis of each line is given in Table 4.

In late 1981, the LC_{50} for chlorphoxim for young larvae at Chutes Gauthier had become eight times higher than in January 1977 and 12 times higher than in February 1981 (Table 1 - Tests 1, 2 and 8 compared with 15 and 16). The upper limit for the LC_{100} increased from 0.0125 mg/l (diagnostic concentration) to 0.05 mg/l. **The slope of the log dose-probit** mortality line did not change significantly.

For mature larvae, the LC_{50} at Chutes Gauthier in October 1981 is about eight times the figure for temephos-resistant larvae (Asserekro Test No. 12, Table 1), and at least 10 times the figures for normal populations (Table 3). **The upper limit of the LC_{100} is 0.5 mg/l** versus 0.03125 mg/l or 0.0625 mg/l for normal populations. **The slopes of two of the log** dose-probit mortality lines are (Nos. 17 and 24) significantly less for the resistant populations. The absolute values for mature larvae are about 10 times those for young larvae (compare line No. 15, Fig. 1 with line No. 14, Fig. 2).

All mature larvae surviving diagnostic concentrations were identified as S. soubrense/ sanctipauli, even when other cytospecies were present in the general population.

By January 1982, the resistance to chlorphoxim covered most of Ivory Coast. All S. soubrense populations in the treated area are now resistant, and the area is the same as for the previous temephos resistance. **Only the Black Volta River in Ghana contains** S. soubrense resistant to temephos and not to chlorphoxim.

However, farther away from the lower Bandama River the resistance is less "intense" as indicated by LC_{50}, LC_{95} and LC_{100} values and percentage of survival at the diagnostic dose. For example, at Grechan (No. 21, Table 2) only 4.1% of young larvae survived at the diagnostic dose versus 70.5% at Chutes Gauthier (No. 16, Table 1). An interesting exception to this trend was seen on the Bafing River (Nos. 16 and 17, Table 2), where 100% of the mature larvae survived versus 73% at Chutes Gauthier (Test 14, Table 1).

Discussion

The existence of well-developed resistance to chlorphoxim in S. soubrense/sanctipauli, suspected because of treatment failure, is confirmed by the **data presented here.** These show large increases in LC_{50}, LC_{95} and the limit of the LC_{100}. **The slopes of the log dose-**probit mortality lines with young larvae are not, however, reduced as they were with temephos resistance (Guillet et al., 1981). This may indicate greater genetic homogeneity.

The data for mature larvae show the same changes as those for young larvae, although the absolute values are higher. **This demonstrates that mature larvae can be used for** diagnostic testing, but that the data must be compared with baseline data obtained using mature larvae. **If tests with both young and mature larvae are included in baseline surveys,** operational flexibility will be gained when follow-up tests are needed.

Operationally significant resistance to chlorphoxim developed in temephos-resistant larvae in a period of five months, according to test results. **Even if the starting point is** considered to be the first chlorphoxim treatments in 1980, resistance took 16 months to develop. **Several factors may have been involved in this rapid development of resistance.** Firstly, the population treated was already resistant to temephos. Since temephos and chlorphoxim are both organophosphorus compounds, it is likely that the same enzyme system would detoxify both compounds and resistance to one would predispose for development of resistance to the other. As there was no evidence of cross-resistance[1] early in the period of chlorphoxim use, additional selection was necessary to develop the double resistance[2] now exhibited by these larvae.

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[1] Cross-resistance is here defined as resistance to an insecticide which has never been applied in a strain already resistant to one or several insecticides.

[2] Double-resistance is here defined as resistance to a replacement insecticide appearing sometime after its introduction.

Secondly, the dosage rate used for chlorphoxim in the field (0.025 mg/l for 10 minutes) was fixed as low as possible since chlorphoxim is more toxic to non-target organisms than temephos (Dejoux & Troubat, 1976). This may have resulted in less than 100% effective treatments which encouraged rapid selection for resistance.

Once selection began, the relative isolation of the lower Bandama population (Guillet et al., 1980) would favour fixation of the genes for resistance. It is not possible, however, to prove that isolation was essential to development of resistance in this case. Most neighbouring populations of S. soubrense/sanctipauli were also resistant to temephos and being treated with chlorphoxim. Thus flies arriving from other sites would have been exposed to the same selection pressure. Also, since most evidence indicates that flies mate before dispersal, flies arriving from non-treated areas would not "dilute" a resistant population in a successfully treated river. Their susceptible genes would be eliminated when their susceptible offspring were killed by the insecticide treatments (Reiter, 1981). If, however, treatments were not 100% effective due to poor distribution of the insecticide, then resistant and non-resistant larvae would have equal chances of survival and dilution would be a factor.

In regard to the question of the geographic origin of the resistance, the data generally indicate a spread by dispersing flies from the lower Bandama River. However, the results on the Bafing may indicate a secondary source.

The data presented here clearly demonstrate resistance to chlorphoxim by S. soubrense/sanctipauli larvae through most of Ivory Coast. Studies are under way to determine the cross-resistance spectrum of resistant larvae and the effects of synergists of the susceptibility of resistant larvae to chlorphoxim.

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TABLE 1. SUSCEPTIBILITY TESTS WITH CHLORPHOXIM IN THE BANDAMA RIVER BASIN, IVORY COAST
(chronologically arranged)

No.	Date	Site and river	Map code	Age of larvae	No. of larvae	LC_{50} (mg/l)	LC_{95} (mg/l)	Upper limit LC_{100} (mg/l)	Ratio LC_{50}/LC_{95}	% survival[a] at diagnostic dose	Conclusion	Cytotaxonomic[b] identification
1	20/1/77	Chutes Gauthier (Bandama)	2	Young	335	0.0015	0.005	0.0125	3.0	0	Normal	75% sa 25% so
2	21/1/77	Chutes Gauthier (Bandama)	2	Young	523	0.0016	0.004	0.0125	2.5	0	Normal	75% sa 25% so
3	4/6/80	Chutes Gauthier (Bandama)	2	Young	128	-	-	0.0125	-	0	Normal[c]	100% sa
4	14/9/80	Danangoro (Marahoué)	5	Young	231	-	-	0.0125	-	0	Normal[c]	100% so[d]
5	15/9/80	Danangoro (Marahoué)	5	Young	620	0.0023	0.0051	0.0125	2.2	0	Normal[c]	100% so[d]
6	13/1/81	Hermis (Marahoué)	7	Young	66	-	-	0.0125	-	0	Normal[c]	98% so 2% da
7	3/2/81	Chutes Gauthier (Bandama)	2	Young	292	-	-	0.0125	-	0	Normal[c]	55% so 45% sa
8	4/2/81	Chutes Gauthier (Bandama)	2	Young	350	0.0011	0.0019	0.0125	1.7	0	Normal[c]	55% so 45% sa
9	11/5/81	Chutes Gauthier (Bandama)	2	Young	72	-	-	0.0125	-	0	Normal[c]	88% sa 8% so 4% da
10	10/6/81	Sites 2 and 3 (Bou)	8	Young	905	-	-	0.0125	-	0	Normal[c]	100% so
11	4/9/81	Asserekro (Kan)	4	Young	51	-	-	0.0125	-	0	Normal[c]	69% so 18% sa 8% da 5% si
12	7/10/81	Asserekro (Kan)	4	Mature	909	0.014	0.035	0.0625	2.5	0	Normal[c]	77% so 14% sa 9% da
13	20/10/81	Chutes Gauthier (Bandama)	2	Mature	1 153	>0.125	>0.125	?	?	65.3	Resistant[c]	87% sa 10% so 3% so/sa
14	21/10/81	Chutes Gauthier (Bandama)	2	Mature	689	0.16	0.36	0.5	2.25	73.5	Resistant[c]	87% sa 10% so 3% so/sa
15	23/10/81	Chutes Gauthier (Bandama)	2	Young	187	0.014	0.060	0.05	4.3	71.1	Resistant[c]	87% sa 13% so
16	24/10/81	Chutes Gauthier (Bandama)	2	Young	434	0.019	0.035	0.05	1.8	70.5	Resistant[c]	87% sa 13% so
17	27/10/81	Chutes Gauthier (Bandama)	2	Mature	215	0.15	1.3	0.5	8.7	61.7	Resistant[c]	88% sa 12% so
18	28/10/81	Kravasso (N'Zi)	3	Mature	215	0.12	0.30	>0.25	2.5	48.5	Resistant[c]	100% so
19	28/10/81	Tiassale (Bandama)	1	Young	88	-	-	>0.0125	-	50.0	Resistant[c]	80% sa 20% da
20	28/10/81	Tiassale (Bandama)	1	Mature	46	-	-	>0.125	-	82.7	Resistant[c]	80% sa 20% da
21	28/10/81	Chutes Gauthier (Bandama)	2	Young	120	0.016	0.03	>0.025	1.9	72.7	Resistant[c]	100% sa
22	15/12/81	Kongasso (Marahoué)	6	Young	115	-	-	>0.0125	-	7.8	Resistant[c]	86% so 10% da 4% so/sa
23	16/12/81	Hermis (Marahoué)	7	Mature	570	0.047	0.14	0.125	3.0	0	Resistant[c]	100% so
24	6/1/82	Latokaha (Bandama Blanc)	9	Mature	372	0.08	0.70	0.25	8.8	26.6	Resistant[c]	100% so
25	6/1/82	Latokaha (Bandama Blanc)	9	Young	26	-	-	>0.0125	-	4	Resistant[c]	100% so

[a] 0.0125 mg/l for young larvae and 0.125 mg/l for mature larvae.

[b] sa = Simulium sanctipauli; so = S. soubrense; da = S. damnosum s.str.; si = S. sirbanum.

[c] Resistant to temephos also.

[d] Laboratory-reared larvae.

TABLE 2. SUSCEPTIBILITY TESTS WITH CHLORPHOXIM OUTSIDE OF THE BANDAMA BASIN
(chronologically arranged)

No.	Date	Site and river	Map code	Age of larvae	No. of larvae	LC_{50} (mg/l)	LC_{95} (mg/l)	Upper limit LC_{100} (mg/l)	Ratio LC_{50}/LC_{95}	% survival at diagnostic dose[a]	Conclusion	Cytotaxonomic[b] identification
1	10/11/81	Bétié (Comoé)	11	Mature	370	0.03	0.19	>0.25	6.3	10.8	Resistant[c]	100% so
2	17/11/81	Akakro (Aguegby)	10	Mature	671	0.014	0.03	0.0625	2.1	0	Normal	100% sq
3	29/11/81	Amouakoukro (Comoé)	13	Mature	418	0.074	0.21	0.5	3.5	24.4	Resistant[c]	100% so
4	30/11/81	Amiakouassikro (Comoé)	12	Mature	615	-	0.15	0.5	-	9.1	Resistant[c]	100% so
5	30/11/81	Amiakouassikro (Comoé)	12	Young	194	-	-	>0.0125	-	28.8	Resistant[c]	100% so
6	1/12/81	Amiakouassikro (Comoé)	12	Mature	609	0.065	0.26	0.5	4.0	20.2	Resistant[c]	100% so
7	17/12/81	Bac Semien (Sassandra)	22	Young	188	-	-	>0.0125	-	4.3	Resistant[c]	100% so
8	17/12/81	Sarakro (Comoé)	14	Mature	652	0.039	0.13	0.5	3.3	5.3	Resistant[c]	100% so
9	17/12/81	Sarakro (Comoé)	14	Young	69	-	-	>0.0125	-	5.8	Resistant[c]	100% so
10	9/1/82	Bui Black Volta	15	Mature	102	-	-	0.125	-	0	Normal[c]	100% so
11	13/1/82	Soubré Sassandra	24	Mature	196	-	-	0.125	-	0	Normal	79% so 18% so/sa 3% da
12	15/1/82	Zobo N'Zo	23	Mature	71	-	-	0.125	-	0	Normal	100% yah
13	19/1/82	Tjokoronidougou Boa	17	Mature	14	-	-	>0.125	-	17	Resistant[c]	69% so 23% yah 8% da
14	20/1/82	Borotou Bagbe	20	Mature	376	0.11	0.26	0.5	2.4	34.1	Resistant[c]	100% so
15	20/1/82	Borotou Bagbe	20	Young	54	-	-	>0.0125	-	44.5	Resistant[c]	100% so
16	21/1/82	No. 1 Bafing	21	Young	99	-	-	>0.0125	-	15.6	Resistant[c]	100% so
17	21/1/82	No. 1 Bafing	21	Mature	51	-	-	>0.125	-	100	Resistant[c]	100% so
18	22/1/82	No. 1 Tyenba	18	Young	34	-	-	>0.0125	-	3.7	Resistant[c]	41% so 50% da 9% si
19	22/1/82	No. 1 Tyenba	18	Mature	113	-	-	>0.125	-	1.0	Resistant[c]	41% so 50% da 9% si
20	22/1/82	Banandougou Bagbe	19	Mature	118	-	-	>0.125	-	1.5	Resistant[c]	84% so 8% sa 8% so/sa
21	26/1/82	Grechan Léraba	16	Young	219	-	-	>0.0125	-	4.1	Resistant[c]	100% so
22	26/1/82	Grechan Léraba	16	Mature	120	-	-	>0.125	-	2.0	Resistant[c]	100% so

[a] 0.0125 mg/l for young larvae and 0.125 mg/l for mature larvae.

[b] sa = Simulium sanctipauli; so = S. soubrense; sq = S. squamosum; da = S. damnosum s.str.; yah = S. yahense; si = S. sirbanum.

[c] Resistant to temephos also.

TABLE 3. SUSCEPTIBILITY TESTS WITH CHLORPHOXIM ON NON-RESISTANT MATURE LARVAE OUTSIDE OF THE BANDAMA BASIN

No.	Date	Site, river and country	No. of larvae	LC_{50}	LC_{95}	Upper limit LC_{100}	Ratio LC_{95}/LC_{50}	Cytotaxonomic identification
1	23/9/81	Yabo, Volta Blanche, Upper Volta	409	0.0076 mg/l	0.015 mg/l	0.03125 mg/l	1.97	100% S. sirbanum
2	17/10/81	Akakro, Agnegby, Ivory Coast	671	0.014 mg/l	0.03 mg/l	0.0625 mg/l	2.14	100% S. squamosum

TABLE 4. STATISTICAL ANALYSIS OF LOG DOSE - PROBIT MORTALITY LINES

Fig.	Line	Site and date	Slope	Standard error of slope	Chi^2 (n =)	p =	LC_{50} (95% confidence interval)	LC_{95} (95% confidence interval)
1	1	Chutes Gauthier 20/1/77	3.15	0.421	1.13 (2)	0.568	0.0015 (0.0012-0.0018)	0.0051 (0.0040-0.0075)
1	2	Chutes Gauthier 21/1/77	4.01	0.348	0.066 (1)	0.797	0.0016 (0.0015-0.0018)	0.0042 (0.0036-0.0050)
1	5	Danangoro 15/9/80	4.71	0.395	3.12 (1)	0.08	0.0023 (0.0021-0.0025)	0.0051 (0.0044-0.0061)
1	8	Chutes Gauthier 4/2/81	6.37	two	data	points	0.0011	0.0019
1	15	Chutes Gauthier 23/10/81	2.63	0.417	5.32 (2)	0.07	0.014 (0.011-0.019)	0.060 (0.038-0.13)
1	16	Chutes Gauthier 24/10/81	6.46	0.81	1.39 (1)	0.24	0.019 (0.018-0.021)	0.035 (0.031-0.043)
2	12	Asserekro 7/10/81	4.033	0.248	11.267 (3)	0.01	0.014 (0.010-0.016)	0.035 (0.027-0.060)
2	14	Chutes Gauthier 21/10/81	4.63	0.427	6.85 (1)	0.009	0.16[a]	0.36[a]
2	17	Chutes Gauthier 27/10/81	1.73	0.579	0.52 (2)	0.77	0.15 (0.12-0.19)	1.3 (0.75-3.4)
2	23	Mermis 16/12/81	3.56	0.281	0.357 (1)	0.55	0.047 (0.042-0.053)	0.136 (0.108-0.189)
2	24	Latokaha 6/1/82	1.76	0.568	9.51 (2)	<0.01	0.081[a]	0.70[a]

[a] Heterogeneity does not permit calculation.

FIG.1 DOSE - MORTALITY CURVES FOR SUSCEPTIBILITY TESTS WITH CHLORPHOXIM IN THE BANDAMA BASIN 1977 TO 1981 (YOUNG LARVAE OF SIMULIUM DAMNOSUM S. L.)

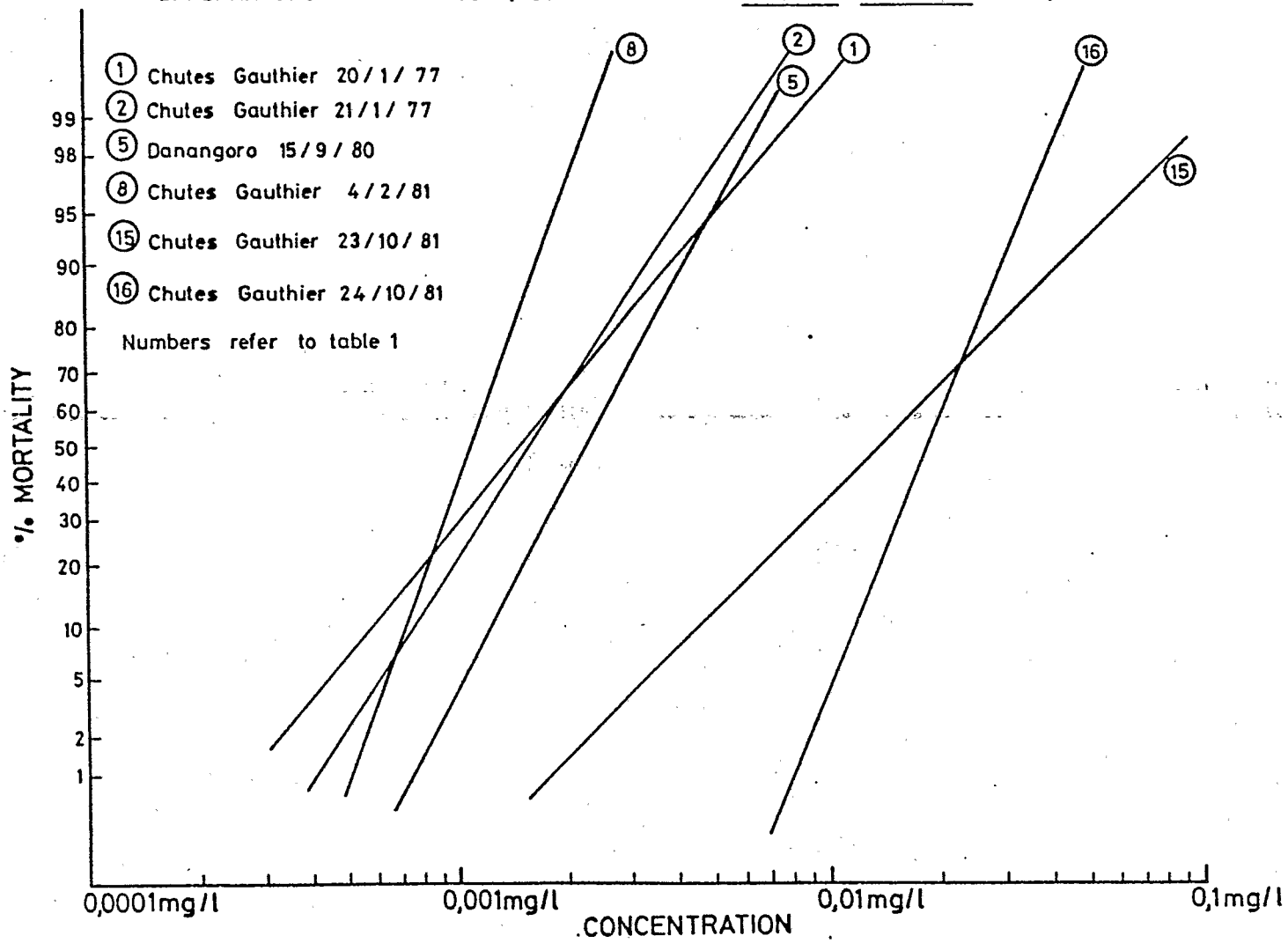


FIG. 2 DOSE - MORTALITY CURVES FOR SUSCEPTIBILITY TESTS WITH CHLORPHOXIM IN THE BANDAMA BASIN (MATURE LARVAE OF SIMULIUM DAMNOSUM S. L.)

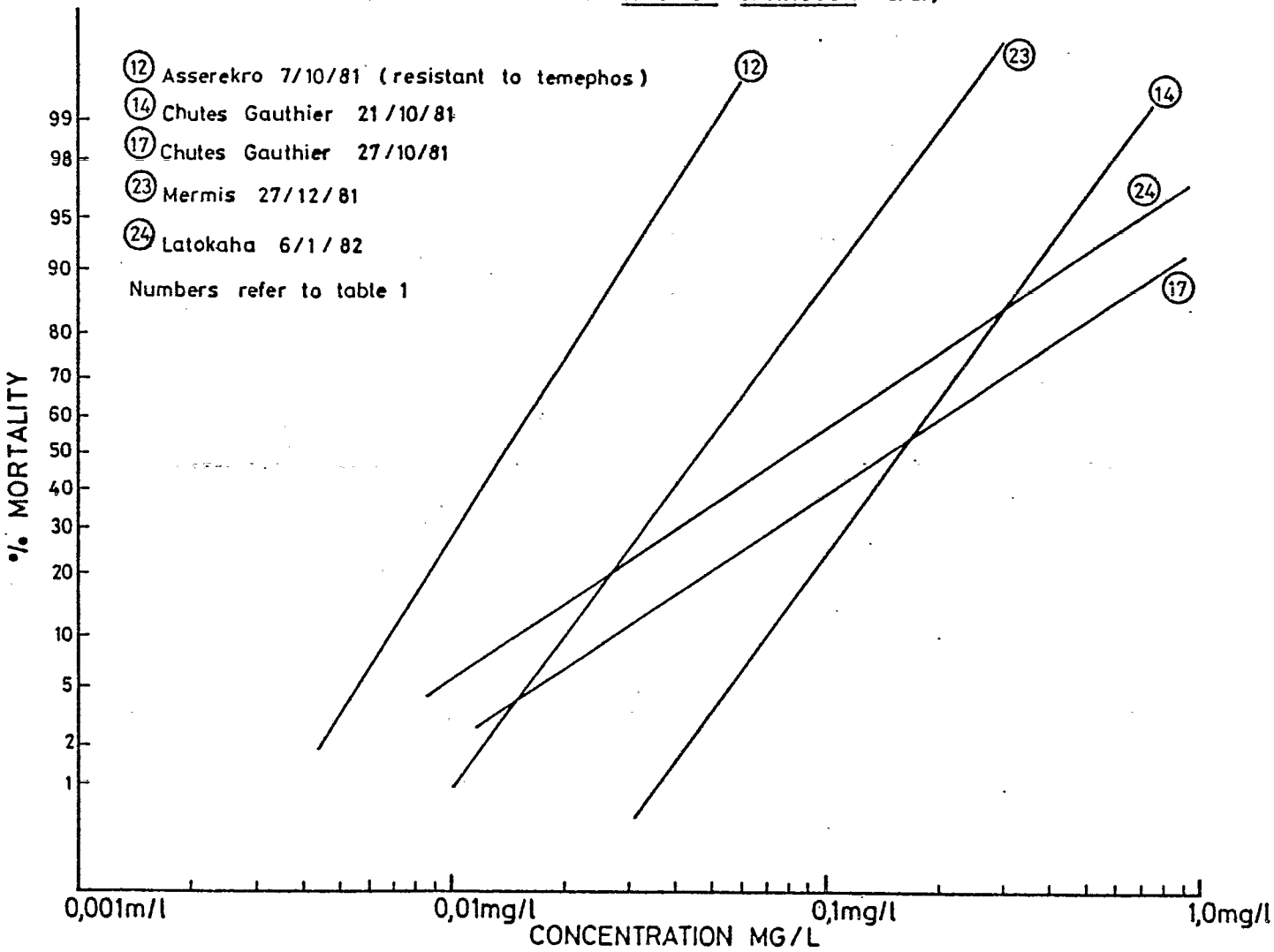
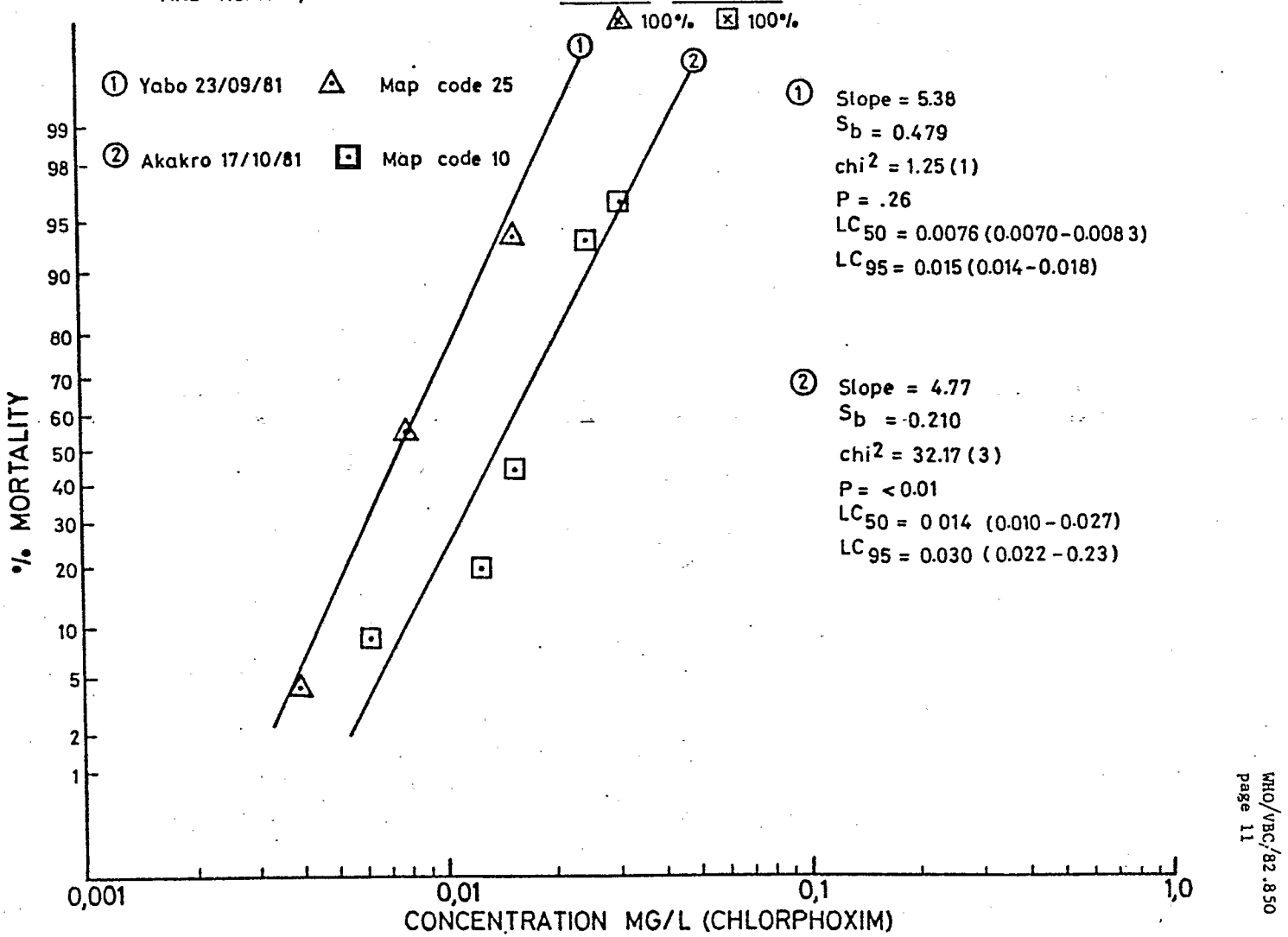


FIG. 3 DOSE - MORTALITY CURVES FOR SUSCEPTIBILITY TESTS WITH CHLORPHOXIM AND NORMAL, MATURE LARVAE OF SIMULIUM DAMNOSUM S. L.



ONCHOCERCIASIS CONTROL PROGRAMME IN THE VOLTA RIVER BASIN AREA

PROGRAMME DE LUTTE CONTRE L'ONCHOCERCOSE DANS LA RÉGION DU BASSIN DE LA VOLTA

FIGURE 4. EXTENT OF RESISTANCE TO CHLORPHOXIM