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SUSCEPTIBILITY AND "NATURAL TOLERANCE" TO INSECTICIDES  
ON THE PART OF LOCAL VECTORS AFTER EIGHT YEARS OF  
APPLICATION OF THE IMAGOCIDE METHOD IN ROMANIA

COMPARATIVE STUDY OF THE VARIOUS TESTS OF EFFICACY AND  
RESIDUAL POWER OF DDT AND EHC WITH THE  
BUSVINE & NASH STANDARD TEST

by

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At the beginning of the third year of the malaria eradication programme in our country (a programme which is at present in the "attack phase"), the constant threat of eventual development of physiological resistance on the part of the local vectors, A.m. atroparvus, A.m. messeae, A.m. typicus and A. sacharovi, is the guiding factor in the continually expanding entomological research for the detection of the slightest sign of this phenomenon.

We would recall that the clearly defined purpose of our antimalaria work is to obtain - as a matter of urgency - the elimination of parasites in all carriers before efforts to interrupt transmission become ineffective as a result of the appearance of physiological resistance on the part of the above-mentioned vectors after eight years of imagocide action by means of chlorinated hydrocarbon insecticides.

I. Susceptibility on the part of local malaria vectors to DDT and lindane insecticides after eight years' use of these substances on the coast and in the Danube valley

by

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Readers will find in a previous note on malaria in Romania<sup>1</sup> some details relating to endemicity and the antimalaria methods employed. In fact, in 1948-49, priority was given, in a first stage, to imagocide activities, using residual insecticides, i.e. DDT in solution or emulsion, applied in a dosage of about 2 g/m<sup>2</sup>, and BHC in solution in a dosage of 0.25-0.30, 0.37 g/m<sup>2</sup> gamma isomer. In addition to the use of these insecticides, modern therapeutic methods were employed to combat the parasite, and systematic treatment was applied to clinical cases and parasite carriers.

As a rule, spraying operations were commenced at the end of April or beginning of May and ended in certain localities in July (or exceptionally in August). In general, one seasonal spraying was sufficient.

For strictly local reasons (work sites, etc.) very small antilarval operations were conducted, using BHC in 0.0025-0.0030 g/m<sup>2</sup> gamma isomer, in two zones only, i.e. the Giurgiu work site (1952-1953), and the Carasu Valley (1954-1955). These operations were carried out at 15-30 day intervals before the appearance of larvae at the advanced stage IV and of nymphs (more resistant to insecticides).

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<sup>1</sup> "Principes et méthodes de lutte antipaludique. Sept ans d'expérience roumaine des pulvérisations insecticides à action rémanente en vue de l'arrêt de la transmission de l'infection." Bulletin de Parazitologia Medicala, Bucharest, 1955, No. 2, p. 3.

Summary in Mal/Inform/7 (28 November 1955).

See also Report of the Second Malaria Conference of the countries of South-East Europe, Belgrade, 26-29 March 1957 (Doc. WHO/Mal/187 EURO-107/2; 29 April 1957).

### Methods of Evaluating Efficacy

The efficacy of the imagocide method was verified and evaluated regularly by:

(a) epidemiological tests

- effectiveness of interruption of transmission;
- spleen and parasite rates in children of 0-14 years during the epidemic season;

(b) evaluation of anopheline density in houses, stables, animal sheds, etc. before and after spraying;

(c) measurement of residual insecticide on treated surfaces (biological test, wall cage);

(d) from 1956, in addition to the above-mentioned control tests, the Busvine & Nash (1953)<sup>1</sup> method of testing the sensitivity of anophelines to insecticides was also added. Comparative tests were made - in so far as possible - according to the technical indications given by the authors (and adopted by the WHO Expert Committee on Malaria) and in the light of local circumstances.

In the absence of laboratory anophelines, the experiments were limited in the first instance to experimental intoxication tests on adult anopheles captured in the field in sprayed localities, and also in non-sprayed localities for control purposes. The distances between the places of capture and the field laboratory varied from 2 to 120 km.

We ourselves carried out a very strict check on anopheline density in 1956, at Eforie, Zebil and Murighiol - localities sprayed for 8-9 years consecutively - and at Agigea, Enisala and Caraibil, localities which were sprayed for 5-7 years consecutively but where spraying had been suspended during the last two years. The average density (established in 10-12 dwellings situated in groups of 2 or 3 at various distances from the larval breeding-places) was calculated on the basis of a count or "estimation" of the anopheles found in the dwellings and animals' quarters.

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<sup>1</sup> We would take this opportunity of expressing our gratitude to the WHO Malaria Section which placed at our disposal two kits containing the necessary material and standardized products for the making of this test.

### Interpretation of Results and Discussion

The anopheline density registered in the period of maximum anopheline development in our country (July-August) in three localities (Agigea, Enisala, Caraibil) where spraying had been suspended in 1956, varied between 83.3 and 542.4 anopheles per habitation, whereas in three other localities (Eforie, Zebil and Murighiol) which had been continually treated for the past 8-9 years, the average varied between 0 and 4.9 anopheles per habitation sprayed with DDT or BHC. Three to four weeks after spraying, nevertheless, a certain number of anopheles were found in some localities (Dunăvățul de Jos and Castelu - continually sprayed), in non-sprayed stables or sprayed animal shelters, these latter constructed entirely of tightly-woven reeds. In these two last-named localities, anopheline mortality after 24 hours, measured 3-4 weeks after treatment, was 10-15% (wall cage) in the sprayed stables, whereas in the living quarters of the same building it remained at 100%.

### Busvine & Nash Method

In testing anopheline susceptibility to insecticides by the Busvine & Nash method, we examined 8584 anopheles (of which 4502 specimens were subjected to experimental intoxication and 4077 served as controls) captured in 24 localities of the coast and Danube valley. The captures were made in sprayed localities (treated and non-treated dwellings), or in localities not sprayed during the last years, or never sprayed at all.

### Results

The figures, corrected according to Abbott's formula, emphasize the following peculiarities:

(1) in the Tîr neighbourhood, which is surcharged with chlorine emanations and residue from chlorinated insecticides (insecticide factory) and at Cusțelnic (control locality 10 kilometres away), the mortality rates ranged between the following extremes:

with 0.02% lindane - mortality 79.9 to 83.3%

with 0.04% lindane - mortality 84.3 to 91.0%;

(2) in the localities of Sărulești, Chirnogi and Cîsla, which were sprayed for 7-8 successive years, the mortality was registered as follows:

with 0.04% lindane - mortality 90.1 to 90.2%

with 2% DDT - mortality 87.0 to 100%;

(3) on the other hand, at Castelu and Dunăvățul de Jos (sprayed continually from 1949 to 1956) the mortality among anopheles captured in some sprayed stables was:

with 0.025% lindane - mortality 70.3%

with 0.04% " - " 70.4 to 93.8%;

(4) the lowest mortality rates (43.7% for 0.02% lindane and 78.9% for 0.4% lindane) were registered at Eforie, which was sprayed without interruption for 9 years. On the other hand, in the control locality (Agigea), sprayed without interruption for 7 years (1948-1954) the mortality registered in 1956, after 2 years' interruption, was 91.0 to 96.6% with 0.02% lindane, and 96.1 to 99.2% with 0.04% lindane;

(5) paradoxically, we noted that the lowest mortality rates with 0.04% lindane were registered in: Isbiceni (65.7% on 20 September 1956), which was partially sprayed in one year only (1953), and Săftica (47.5% on 15 November 1956), which was never sprayed at all;

(6) the presence of a large number of anopheles in some sprayed stables at Dunăvățul de Jos and Castelu (see under (3)) does not seem to have been due to the appearance of the resistance phenomenon, but rather to a lack of residual power in the insecticide used in the treated stables. Neither do the low mortality rates at Eforie (43.7% with 0.02% lindane and 78.9% with 0.04%) seem to have been caused by any resistance factor seeing that in this locality the anopheline density was nearly always nil in sprayed dwellings in 1956, and the small number of anopheles captured during one of these experiments came from a single control dwelling which had not been treated with insecticide.

Summing up, it may be said with respect to the coastal and Danube valley areas, where there was high endemicity before 1948, that the following facts emerge:

- (a) low level of anopheline density in the localities sprayed without interruption;
- (b) similar mortality rates among captured anopheles subjected to the Busvine & Nash intoxication test (whether from continually sprayed localities, or from localities where spraying had ceased for two years, or from control, non-sprayed localities);
- (c) absence of malaria in the localities treated with insecticide for a certain number of years without interruption.

These facts seem to indicate that the local mosquito vectors studied are still maintaining their susceptibility to the insecticides.

Other corroborating facts appear to be: reduced anopheline density registered at Eforie (locality sprayed for 9 years without interruption); lower mortality rates (43.7% with 0.02% lindane and 78.9% with 0.04% lindane) in this same locality, and similar rates at Isbiceni (partially sprayed once only in 1953 and where a mortality rate of 65.7% with 0.04% lindane was established) and at Săftica, a locality which was never sprayed at all and which gave a mortality rate of 47.5% with 0.04% lindane.

Obviously, in all the localities where experiments were carried out on a limited number of anophelines only, the investigations must be continued in order to detect any possible seasonal variations in sensitivity of the anopheles to the same insecticides in the same concentrations.

As soon as we have our own colonies of anopheles available - in 1957 - large-scale investigations will also be made with respect to variable factors such as age, feeding, temperature, humidity, etc. Only by creating strict experimental conditions shall we be able to draw definite conclusions with regard to the behaviour of our vectors after an eight-year imagocide campaign.

II. Preliminary observations relating to sensitivity to DDT and EHC on the part of anopheles of the maculipennis group in North Moldavia

by

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A small-scale preliminary test carried out in 1953/1954 in certain sprayed localities seemed to indicate that there was less sensitivity to DDT and EHC than in non-sprayed localities.

In this connexion, we would mention that the localities in question were in the Jijia and Bahlui valley, whose principal characteristics are: presence of stagnant water constituting larval breeding-places in upland zones of altitudes ranging from 30-50 m to 400 m; wooded steppe and pre-steppe climate; in hilly districts the climate undergoes modifications which are related to the vast wooded areas. By order of importance, the local anophelines are A. maculipennis, A. messeae and A. atroparvus; in the hilly districts, the percentages of A. maculipennis vary between 75 and 90. In general, five generations of anopheles are produced during hot years.

From 1956, co-ordinated research was carried out: (a) applying the Busvine & Nash method to 9074 intoxicated anopheles, and 4911 controls; (b) by means of a microtest, using the Ungureanu ultra-micropipette, on 1619 intoxicated anopheles and 85 controls kept in natural conditions.

For the microtest with the Ungureanu pipette, alcohol solutions of 0.1%, 0.5% and 0.25% DDT were used, and we took into account the adjustments to be made to compensate the temperature variations. The mosquitos, anaesthetized with ether, were placed on a slide so that the drop of insecticide could be applied to the dorsum of the thorax. Duration of anaesthesia: 2 minutes after evaporation in paper cages.

Insecticides Used

From 1947 to 1956, all the localities were sprayed with DDT at the rate of 2-2.5 g/m<sup>2</sup> and EHC at the rate of 0.25-0.30 g/m<sup>2</sup> gamma isomer, some localities without interruption.

In the spraying conditions established in north Moldavia, the effects of the insecticide were noted even on the first generation in some localities while in others the effect was noticeable from the second or third, and sometimes fourth, generation; 80% to 90% of shelters were sprayed and only the imagocide method was used - (no anti-larval measures).

For purposes of observation, larger paper cages (Ungureanu model) were used so as to ensure sufficient space and more favourable conditions for the mosquitos than with the usual gauze and wire cages. The paper cages were used once only,

Some of the experimental intoxication tests were carried out in the place of capture, some in the laboratory insectarium.

No intoxication tests were made at a distance of more than 15 km; the anopheles were transported in 25 x 25 x 35 cm cages.

Precision instruments were used for the constant registration of temperature and humidity during the experiments.

The captured adult anopheles came from sprayed and non-sprayed localities in the neighbourhood - which did not exclude the possibility of mixed anopheline fauna.

Reading of the results took place in general after 24 hours and after 48 hours, in most of the experiments.

The average figures, with the Busvine & Nash method, were as follows:

	<u>Concentration</u>	<u>Mortality %</u>	
		<u>Min.</u>	<u>Max.</u>
DDT	1%	12	56
	2%	19	76
Lindane	0.02%	19	36
	0.04%	41	80

In some rare cases, results of over 90% were noted (for example in Podgoria, locality sprayed once only, in 1956, surrounded by localities sprayed during preceding years).

The results obtained for DDT with the microtest show that in this region A. maculipennis mortality was more than 90% using 0.540 gamma, whereas it was only 64% with 0.300 gamma.

#### Discussion of Results

Bearing in mind the methods employed and the selective power of the resistance process, it would seem that in our region selectivity has been inconstant and partial.

The data so far obtained are insufficient for the drawing of conclusions with regard to the degree of anopheline susceptibility to, or tolerance of, DDT and BHC, or with regard to the extent to which there may be hereditary resistance. If this latter exists, it is so slight that it cannot prejudice the effectiveness of the antimalarial campaign by means of insecticides, in the concentrations used in our country.

Epidemiological and biological tests show that malaria has disappeared from the zone as a result of the imagocide campaign.

On the basis of experience acquired in 1956 in north Moldavia, the following points may be stressed:

There is a "natural non-specific resistance" which depends upon the species of insect involved. In fact, doses which bring about 100% mortality of Stomoxys calcitrans do not cause more than 50% mortality of Musca domestica although there is no very great difference in the size of these insects. On the other hand, the laboratory Musca domestica gives an individual mortality rate of 90% with 2 gamma, whereas A. maculipennis - even in sprayed zones - needs only 0.300 gamma per specimen to obtain 90% mortality.

Ixodes ricinus, Dermacentor marginatus, Haemophysalis, etc. require doses 10-20 times that used for Musca domestica, in order to obtain 90% mortality.

"Growth" factors play a very important role in the response of anopheles to insecticides: a certain degree of "tolerance" is noted in September-October even in non-sprayed localities (Busvine & Nash test, and microtest).

Furthermore, it is a well-known fact that "last generation" anopheles are much more resistant to unfavourable laboratory conditions than are the earlier (summer) generations.

It will, therefore, be seen that in order to be able to assess the degree of susceptibility to insecticides in a locality, research must be conducted from spring-time up to late autumn (April to October). Only in these conditions can any accurate comparison of results be made.

It will be noted that in a non-sprayed locality, and during the period 20 June 1956 - 25 October 1956 the mortality caused by 2% DDT, using the Busvine & Nash test, varied from a maximum of 76% to a minimum of 19%. The maximum number of survivals was noted in September and October.

Analysing the variations in physiological conditions during the anopheles' "pre-hibernation" period - in connexion with the last investigations made by Mer & Furmaska, and by Wiesmann, on the subject of Musca domestica - it would seem that the presence of some fatty substance might be a factor encouraging the natural resistance observed during the autumn.<sup>1</sup>

This phenomenon, moreover, was noted in north Moldavia, in all the localities under observation.

These facts confirm the view of the entomologists of north Moldavia when they state that there exists "in the normal state" an increased resistance to toxic effects in "last generation anopheles" which have never previously been in contact with the insecticide.

This "non-specific natural resistance" is modified according to the conditions of development of the generation in question and according to the living conditions of the adults.

The authors insist upon the importance of reading the results again after 48 hours. Maximum mortality after 24 hours and reduced or similar rates after this interval seem to indicate some mechanism of defence against the insecticide.

To sum up, it may be said that in spite of the variability of the 1956 results, we may hazard the statement that in north Moldavia there is a small number of anopheles showing a relative degree of resistance to DDT and BHC, although the phenomenon is barely noticeable or very slight.

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<sup>1</sup> See References

These results support the statement made above to the effect that local anopheline fauna, predominantly A.m. maculipennis, consists of a mixture of non-resistant individuals which outnumber a smaller group of resistant individuals. This fact has been demonstrated by both of the methods used.

The degree of resistance noted by no means renders ineffective the imagocide measures which have been adopted for the purpose of interrupting the transmission of malaria.

### General Conclusions

In view of the fact that malaria incidence in 1956 was reduced to 154 sporadic cases or microfoci at the beginning of the third year of the "attack phase" of a malaria eradication programme, extensive research work was carried out in 1956 to discover the degree of susceptibility to insecticides on the part of local vectors A. sacharovi, A.m. atroparvus, A.m. messeae, A.m. typicus, after an 8-year imagocide campaign with DDT and BHC for the purpose of interrupting transmission.

As criteria for the evaluation of the efficacy of the method, we used on the one hand biological and epidemiological tests, checking of residual insecticides effect in relation to anopheline density, and experimental intoxication in wall cages and, on the other (in 1956) the verification of these tests by the standard Busvine & Nash method applied to:

- (a) 13 581 captured adult anopheles  
8988 control specimens kept in natural conditions
- (b) 1619 captured adult anopheles subject to the intoxication microtest  
886 anopheles used as controls, in natural conditions.

Two facts emerge:

1. The results of the tests used on a permanent basis and of the Busvine & Nash test tally completely, so that it may be affirmed:

- (a) that the local vectors are still susceptible to DDT and lindane;
- (b) that up to the present time, the development of the malaria eradication programme is not affected by any modification in the degree of susceptibility to insecticides on the part of the vectors.

2. There are regional variations in the life cycle of the vectors and the environmental factors play a role (conditions of field operations, number of generations, age, temperature, humidity, conditions under which the anopheles are transported, etc.). These variations already indicate (more particularly in north Moldavia) some degree of "natural tolerance" both in sprayed localities and also in control localities which have never been in contact with insecticides.

These facts call for an extension in 1957 of research by a parallel method using anopheles captured in the field and laboratory anopheles, of clearly defined age and environment.

We are paying particular attention not only to north Moldavia but also to one of twenty-four coast localities (Eforie) where there is a higher percentage of "survivals" after the Busvine & Nash test in spite of the absence of any epidemiological sign of interference, seeing that no case of malaria has been found there and that the anopheline density in treated houses is 0-0.3%.

We propose to continue investigations in 1957 on all generations of anopheles taken in the field from April to October, as well as on colonies originating from local ovipositions.

In view of the fact that malaria incidence has been reduced to 15<sup>4</sup> sporadic cases (or microfoci) registered in regions of very low endemicity or free from malaria in the past, in which focal or barrier spraying of insecticides commenced only in 1954 or 1955 but which, nevertheless, are threatened by the possibility of development of physiological resistance, we propose, from 1957, to stop the total spraying operations in all localities where examination of the population shows that the parasite has been eliminated.

In conclusion, and in accordance with the recommendations of the Sixth Report of the Expert Committee on Malaria,<sup>1</sup> the malaria eradication programme will be considered as having attained its object only if, during three years of strict epidemiological control, with suppression of all insecticide treatment during the last two years, no sign of transmission or of residual endemicity has been noted.

<sup>1</sup> Document WHO/Mal/180, 28 June 1956

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