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Field Study of the Safety of Abate for Treating Potable Water and Observations on the Effectiveness of a Control Programme involving both Abate and Malathion*

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Abate is a larvicide for Aedes aegypti, *a vector of yellow fever, dengue, and haemorrhagic fever. It is less toxic than malathion or DDT. Tests in human volunteers had previously indicated it would be safe to add to drinking-water where the mosquito often breeds.*

In a 19-month test in Puerto Rico, Abate was added to the drums and cisterns in which a community of about 2000 persons stored their drinking-water. This use of Abate and other insecticidal measures (malathion spraying) gradually achieved control of A. aegypti *during the first 4 months of treatment and control was maintained thereafter although the test area was surrounded by active breeding-sites. There was no accumulation of Abate in the water in spite of monthly additions of the compound. With minor exceptions, residents readily accepted this use of Abate. Careful surveillance failed to reveal any illness or significant side-effects attributable to measures adopted in the control programme. Abate is considered safe for full-scale field use according to directions.*

The organic phosphorus compound, Abate (*O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate) (OMS-786), produced neither clinical ill-effects nor detectable changes in blood cholinesterase levels when given under controlled conditions to volunteers at a dosage of 64 mg/man/day for 30 days (Laws et al., 1967). In view of these findings and of the results of toxicity experiments in animals (Gaines, Kimbrough & Laws, 1967), it was considered safe to undertake a limited field trial in an area where larvicidal treatment of open barrels of stored drinking-water was necessary to achieve effective mosquito control.

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It has been demonstrated that treatment of such stores of drinking-water with Abate sand granules at the nominal rate of 1 ppm is highly effective for controlling the larvae of *Aedes aegypti* (Brooks, Schoof & Smith, 1965). It has also been shown that not all of the Abate added to water is mobilized. The compound hydrolyses slowly so that the highest concentration of Abate reached, following a single application at the recommended rate, is about 0.5 ppm. Even when drums of water were re-treated at 3- and 6-week intervals at a nominal rate of 1 ppm, the Abate concentrations in the water did not increase appreciably above 0.5 ppm (Brooks et al., 1967). Thus, the maximum dosage received by people who drink water at the usual rate of 2 litres per day is about 1 mg/man/day if the water is treated with Abate according to directions.

Because Abate had not been used previously for control of mosquitos in drinking-water, it was considered a good idea to maintain medical as well

as entomological surveillance during its introduction for this purpose. Through the active participation of official health agencies, an attempt was made to learn of any unusual illness among the hundreds of participants in the study. In addition, more detailed attention was given to a small number of volunteers, who were willing to give periodically all the samples of blood and urine the laboratory was prepared to analyse. Chemical studies of these samples involved the known effects of Abate, and the observers were especially alert for any clinical effects characteristic of organic phosphorus compounds even though no effect of this or any other kind was expected.

METHOD

The necessary clearance for the study was obtained from the Puerto Rico Department of Health. An area called Cerro Gandía, located on a high hill, in the town of Manatí, Puerto Rico, was selected as the site of the field trial. During the first 16 months of the study, the area was served by 5 exterior public faucets. However, the water pressure was intermittent and frequently low, and, as a result, much of the drinking-water was either rainwater stored in cisterns or water transported from lower ground and stored in open drums or barrels outside the dwellings. The study area comprised the equivalent of about 9 square blocks. It had 401 premises and a population of approximately 2000 persons. At the beginning of the study, drinking-water was stored in 190 metal drums, 27 wooden barrels, and 10 cisterns. During the 16th month of the study, a 4-inch (10-cm) water main was connected to the existing water line, and one faucet was added so that there were then 6 faucets and enough water pressure to make the supply effective. Considering the placement of the faucets, the new system was capable of servicing conveniently about two-thirds of the study area. Just prior to the study, the index of *Aedes aegypti* infestation in outdoor containers in the 9-block area was found to be positive in 41% of the premises.

Local health authorities and public health nurses were briefed on the protocol of the field trial and on the symptoms and treatment of organic phosphorus poisoning. Residents of Cerro Gandía were told they were free to refuse treatment of their water if they wished.

Initially, 20 volunteers were requested for the donation of blood and urine samples. They received no rewards and were free to discontinue participation at any time. The volunteers, who were obtained without difficulty, were screened for a history of illness. The results are summarized in Table 1.

TABLE 1
POSITIVE FINDINGS ON MEDICAL SCREENING OF 38 VOLUNTEERS PRIOR TO LARVICIDAL TREATMENT

Finding	No. of cases
Allergy to detergents	1
Arthritis	1
Asthma	1
Congestive heart failure	1
Frequent headaches	3
Hypertension	4
History of kidney disease	3
Night sweats	1
Pregnancy	4
Tuberculosis under treatment	1
Varicose veins	1

No volunteer was rejected on the basis of medical history. Because the group studied earlier was composed of adult males only (Laws et al., 1967) the Manatí group was purposely selected to include females primarily and to cover a broader range of ages. The volunteer group eventually totalled 37 women and 1 man, with ages ranging from 15 to 83 years. The average age was 36; the median age, 31. Four of the women were known to be pregnant. The distribution by age-group was as follows: 15–20 years, 6 women; 21–30 years, 8 women; 31–40 years, 9 women; 41–50 years, 4 women; 51 years or more, 10 women and 1 man. Turnover in the volunteer group was the result of moving away from the area, lack of availability of the volunteer for sampling, or knowledge that the volunteer was not drinking treated water. No volunteer had to be dropped because of illness or medical complaints.

All the Abate used to prepare the granules was identical to that employed in the study reported earlier (Laws et al., 1967).

The scheduling of the various procedures in the field study was as follows.

Insectical treatment of the area

All drums, barrels, and cisterns containing potable water were treated with a formulation of 1%

Abate adsorbed on sand, which, if all the Abate were released into 50 US gallons (188 litres) of water, would produce a concentration of 1 ppm of Abate (i.e., 18.9 g of sand granules per drum). This treatment was repeated at monthly intervals from December 1965 through July 1967 except that the 13th monthly treatment in January 1967 was omitted. The rate of addition of Abate was determined by the size of the container; no account was taken of the amount of water which happened to be present at the time of each treatment. In addition, all premises were sprayed and all containers of non-potable water in the study area were treated with 2.5% malathion emulsion at intervals of 2 months. The Cerro Gandía area was sprayed by airplane with technical-grade malathion at the rate of 3 US fluid ounces per acre (about 220 ml/ha) once only at the end of the first 6 months of the study (June 1966). It should be emphasized that no additional measures, such as clean-up and source reduction programmes or screening or covering of drums, were used to supplement control, and no systematic control—chemical or otherwise—was practised in the surrounding area.

Toxicological and medical surveillance

Samples of blood (5 ml–10 ml of whole blood obtained by venepuncture in heparinized tubes) and urine (100 ml or more) were obtained prior to beginning treatment and at intervals of 1, 2, and 4 weeks, 6 months, and 1 year after beginning the treatment schedule. Additional urine samples were obtained at monthly intervals 15 days after each Abate treatment. These samples of blood and urine were analysed at the Toxicology Laboratory, Pesticides Program, National Communicable Disease Center, Atlanta, Ga., USA.

The blood samples were analysed for red blood cell and plasma cholinesterase by automated potentiometric titration at a constant pH, a modification of the method of Jensen-Holm et al. (1959).

Urine samples were analysed for ether-extractable organic phosphorus, and the results were expressed as Abate-equivalent based on the molecular weight and phosphorus content of Abate (Mattson & Sedlak, 1960). The lower limit of sensitivity of the method was 0.05 ppm.

The medical staff and nursing units of the local public health unit and municipal hospital were advised of the study and alerted to report any cases of suspected organic phosphorus poisoning, any unusual incidence of any disease or condition, or any unusual disease or symptom complex occurring in the inhabitants of the study area. The local office of vital statistics was also contacted and it co-operated in this aspect. The health unit, municipal hospital, and office of vital statistics were visited at least once a month by one of the directors of the study. In addition, frequent inquiries were made among the participants in the study.

Chemical analysis of water

Samples of water were taken from 20 of the treated barrels 48 hours after treatment with Abate. These samples were analysed for concentration of Abate at the Technical Development Laboratories of the National Communicable Disease Center, Savannah, Ga., USA.

Upon receipt of the water samples from the field, 300-ml aliquots were extracted three times with 25-ml portions of chloroform and the extracts were combined. After evaporation of the solvent, the Abate content was determined by oxidation of the residue with perchloric acid and colorimetric determination by the molybdenum-blue method or by hydrolysis of the Abate with methanolic KOH and measurement of the absorbance of the resulting 4,4′-thiodiphenol at 263 nm. In some cases, contaminants in the water interfered with the analyses, making a clean-up procedure necessary to obtain reproducible results. The most satisfactory clean-up procedure was to spot the total extract on a thin-layer plate of silica gel, develop the chromatogram with hexane-ether solution and cut out the Abate spot for quantitative determination.

Biological evaluation

The entire study area was inspected for *Aedes aegypti* infestation every 2 months. Both exterior and interior surveys were made. The index for each was based on the percentage of premises found infested.

RESULTS

Toxicological and medical surveillance

No significant change was observed in either plasma or red blood cell cholinesterase at any time during the study; the results of all determinations were within the normal limits.

The results of the analysis of urine are given in Fig. 1. It appears that equilibrium was reached after about 20 weeks of dosage. The Abate-derived compound in the urine dropped rapidly to near control

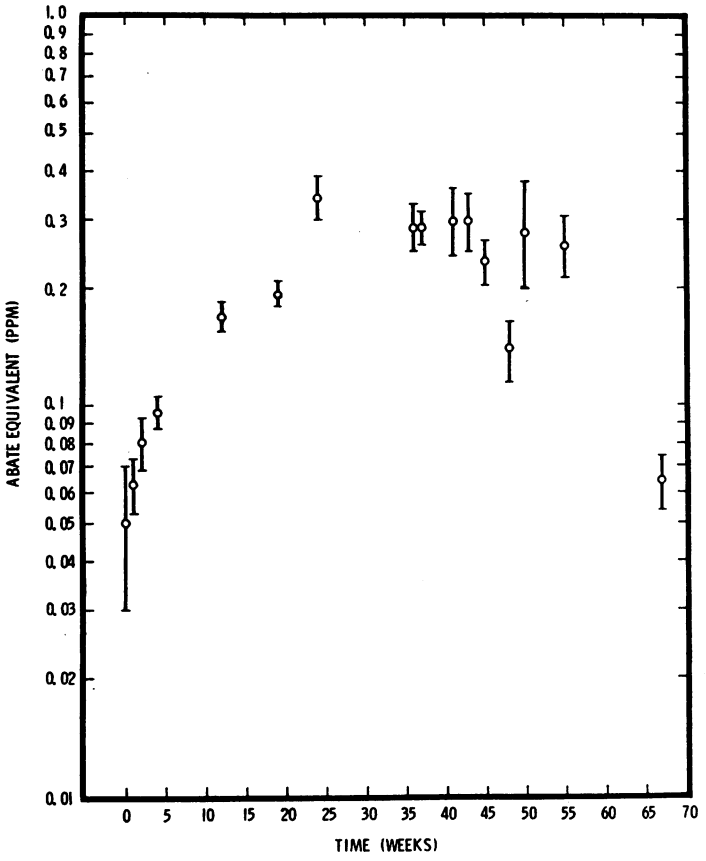


FIG. 1
URINARY CONCENTRATION OF ABATE EQUIVALENT (ppm) IN PEOPLE WHOSE WATER SUPPLY WAS TREATED WITH THIS COMPOUND [a]

[a] The points represent means and the bars represent 1 standard error.

levels after the newly installed water supply provided untreated drinking-water to most of the volunteers. An apparently significant drop in the equilibrium urine levels occurred in early November 1966. This is probably explained by the documented heavy rainfall (4–5 inches, or about 100 mm–130 mm, above normal) in late October, which was enough to reduce the concentration of Abate in the containers.

Because the municipal water supply was changed in March 1967, very few of the original volunteers continued to drink water treated with Abate. Therefore values for urine samples taken after April 1967 have little reliability or scientific value, and they are not reported. It should be noted, however, that the people continued to store water in barrels that continued to be treated with Abate. Thus, data concerning chemical analysis of water samples and infestation of containers by larvae remained valid, and medical surveillance was continued throughout the period in which any water was treated with Abate.

At no time during the study were there any serious illnesses or medical complaints among the volunteer group. Eight babies were born to members of the volunteer group, and all were normal. Four of these babies were conceived and delivered during the Abate study.

Monthly checks with the local public health unit, municipal hospital, and office of vital statistics revealed no change in the birth or death rate, number of spontaneous abortions, or number of stillbirths. No known congenital abnormalities occurred during the study, nor were there any unusual illnesses or deaths among the residents of Cerro Gandía. At least 42 children were born in Cerro Gandía during the study, and at least 22 were conceived after treatment with Abate had begun. The pregnancies and deliveries were normal; the babies were normal; and no neonatal deaths were recorded.

One stillbirth involving a woman who lived just outside the study area occurred in August 1966. The mother, a 24-year-old woman, had had two normal deliveries followed by two spontaneous abortions prior to the study. She lived at the foot of the hill directly opposite an operable public water faucet and denied ever drinking Abate-treated water.

At scattered times during 16 months of treatment, 32 individuals out of the population of about 2000 refused Abate treatment of their water drums. The reasons given for refusal are summarized in Table 2. All but 6 of these people refused only

TABLE 2
REASONS GIVEN FOR REFUSAL[a] OF ABATE TREATMENT OF DRUMS DURING 16 MONTHS OF TREATMENT (200 PREMISES)

Reason given	No.
Allergy	5
Asthma	1
Stomach trouble	10
Diarrhoea	2
Bad taste and/or odour	8
No reason	6
Total refusals	32

[a] All but 6 are refusals of 1 or 2 treatments only out of the total of 16 treatments.

1 or 2 of the 16 treatments. The 4 people who refused more than 3 treatments were interviewed at the end of the study. One was a 47-year-old woman who had had asthma and multiple food allergies since childhood; she was able to link drinking Abate-treated water with fatigue only, and not with symptoms of either asthma or allergy. Another woman said she had stomach cramps once after drinking the water, but she refused all further treatments. A 38-year-old woman developed a pruritic confluent rash on her upper arms and torso after treatments had been in progress for 6 months. However, the rash still persisted 6 months after she refused further Abate treatment of her water drums. Another woman gave diarrhoea as her reason for refusing treatment of the water, but was unable to relate any convincing description of illness when questioned.

All complaints of bad odour of the water were checked by inspectors, but none was confirmed.

There were no complaints of unusual illnesses or deaths among livestock, which included chickens, dogs, pigs, and goats.

Chemical analysis of water

The results of analysis of water samples taken during the test period are summarized in Table 3.

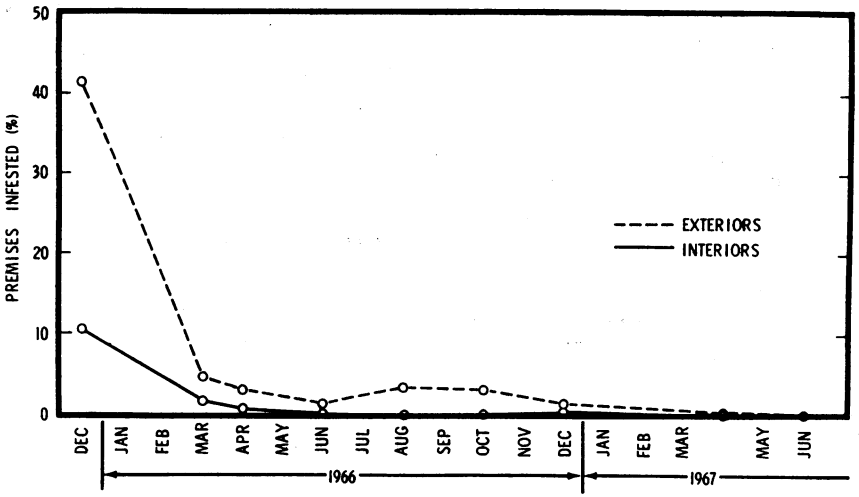
TABLE 3
ABATE CONCENTRATIONS FOUND IN SAMPLES TAKEN IN MANATÍ TEST AREA

Month of treatment	Abate concentration found (ppm)		Method of analysis[a]
	Highest value	Average value	
1 January 1966	0.18	0.03	P
2 February 1966	0.17	0.02	P
3 March 1966	0.12	0.01	UV
6 June 1966	0.74	0.19	UV
10 October 1966	0.05	0.01	P-C
11 November 1966	0.05	0.02	P-C
12 December 1966	0.17	0.06	P-C
15 March 1967	0.46	0.11	P-C
16 April 1967	0.13	0.04	P-C
17 May 1967	0.15	0.06	P-C
18 June 1967	0.25	0.08	P-C
19 July 1967	0.03	0.02	P-C

[a] P = microphosphorus; UV = ultraviolet; P-C = microphosphorus after clean-up on thin-layer plates.

Although considerable variation was observed within each set of samples as well as from one month to another, the data clearly show that no excessive level was reached and no progressive build-up of Abate in the water reservoirs occurred even though the treatments were repeated for 19 consecutive months, with the exception of the 13th month. The data indicate that the water concentrations reflected the amount of rainfall, with higher concentrations appearing during dry periods. In no case was the nominal treatment concentration, 1.0 ppm, observed in any water sample. In fact, only one water sample was found to contain more than 0.5 ppm during the test period.

FIG. 2
PERCENTAGE OF PREMISES POSITIVE FOR LARVAE OF *AEDES AEGYPTI* IN THE AREA TREATED WITH ABATE



Biological evaluation

The index of *Aedes aegypti* infestation throughout the study is given in Fig. 2. It should be emphasized that Cerro Gandía is a geographically distinct area in the midst of a large town which did not receive any planned insecticidal treatment. Reinfestation of Cerro Gandía by *Aedes aegypti* was constantly possible. In spite of this, the exterior index in Cerro Gandía remained quite low; the interior index remained essentially at zero after the first week of treatment; and the over-all infestation index reached zero after 18 months of treatment.

Control was gradually achieved during the first 4 months of treatment. Minor reinfestation of some of the approximately 217 Abate-treated drums and barrels did occur after that period, but never in more than 3 drums during any given inspection cycle. Undoubtedly, some drums were missed during treatment. Occasionally, people acquired new drums, not previously treated, which might serve as a source of reinfestation until treated with Abate.

RÉSUMÉ

Au cours d'un essai pratique d'une durée de 19 mois organisé dans une collectivité de 2000 personnes à Porto Rico, de l'Abate adsorbé sur sable, à la concentration de 1 partie par million, a été ajouté à l'eau de boisson contenue dans les citernes et les réservoirs. Les applications ont été répétées chaque mois de décembre 1965 à juillet 1967. En outre, tous les locaux et les réservoirs d'eau non potable ont été traités par pulvérisations de malathion en émulsion à 2,5% à intervalles de deux mois et on a effectué un épandage aérien unique de ce même insecticide. Des analyses régulières d'échantillons de sang et d'urine prélevés sur des volontaires ont été effectuées en vue de rechercher les effets toxiques éventuels sur la population.

Aucun cas de maladie ou d'effets secondaires pouvant être attribués à l'emploi de l'Abate ou du malathion n'a été observé. On n'a pas noté de modification appréciable du taux de la cholinestérase sanguine, et les dosages du phosphore organique dans l'urine ont montré qu'un équilibre était atteint après 20 semaines environ d'applications. Trente-deux personnes seulement ont refusé à l'un ou l'autre moment de faire usage de l'eau traitée par l'Abate, les raisons invoquées n'étant guère convaincantes; le reste de la population a accepté facilement de la consommer. Les analyses chimiques de l'eau n'ont décelé ni teneur excessive en Abate ni accumulation progressive de l'insecticide en dépit de la répétition des applications. La concentration nominale initiale de 1 partie par million

n'a été notée dans aucun échantillon; un seul d'entre eux contenait plus de 0,5 partie par million d'insecticide.

L'élimination du vecteur *Aedes aegypti* a été obtenue progressivement durant les quatre premiers mois de traitement et s'est maintenue malgré la présence à la périphérie de la zone traitée de gîtes larvaires en activité.

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