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Attempts to Eradicate Snails from Impounded Water by the Use of *N*-Tritylmorpholine

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*This paper records attempts to overcome the problems of using a powerful but non-ovicidal molluscicide (*N*-tritylmorpholine) in eradicating snails from a dam by treating the water twice in 14 days. The first treatment was aimed at the existing snail population and the second at snails hatched from eggs that survived the first application. At each treatment an over-all concentration of 0.25 ppm was applied by spraying a 16 % emulsifiable concentrate of* N*-tritylmorpholine from shore and boat to the water surface. The snails,* Biomphalaria pfeifferi *and* Lymnaea natalensis, *were first seen again 8 weeks from the start of the application in the region of 2 inlet furrows. When pretreatment levels of snails were again reached, a second double treatment was applied, again at an over-all concentration of 0.25 ppm but using a 50 % paste formulation applied over 3 days for 3 hours per day to the inlet furrows. The snail kill was again 100 % as measured by the sampling technique used but again young snails appeared 9 weeks later. The author concludes that single treatments at 5-week intervals probably represent the best means of control with* N*-tritylmorpholine.*

N-Tritylmorpholine (Frescon) is a very specific and very toxic molluscicide. Laboratory screening shows that against *Biomphalaria* spp. the chemical will kill 100% of snails when concentrations of 0.02 ppm–0.05 ppm are used with a 24-hour exposure period. However, it is reported that snail eggs show a marked resistance to the chemical. Paulini & Camey (1964) reported that while adult *B. glabrata* were killed by 0.082 ppm the egg masses had an LC_{90} of 0.023 ppm for a 24-hour exposure, and against *B. sudanica* Webbe & Sturrock (1964) found an LC_{90} of 0.078 ppm for adults but there was no ovicidal activity between 0.1 ppm and 1.0 ppm for a 24-hour exposure.

For field use, Paulini & Camey (1964) suggested 0.2 ppm–0.4 ppm in still water while Crossland (1967) killed most *B. pfeifferi* after 5 days of a 30-days treatment at 0.025 ppm in an irrigation system in Tanzania.

For eradication there are two possible methods—firstly, to keep the molluscicide in the water for sufficient time to destroy all hatching young, and, secondly, to use 2 applications with an interval between them such that all surviving eggs will have hatched before the second application but that none of the hatchlings will have reached the egg-laying stage. In practice this appears to be 14–21 days depending on temperature. The first method was tried by Crossland (1967) when he applied FX 28 (an emulsifiable concentrate of *N*-tritylmorpholine) to give a blanket coverage of 0.025 ppm of the compound to an irrigated estate for 30 days. He obtained effective control for 3–4 months, but as most of the adult snails (*B. pfeifferi*) had died after 5 days, 5/6ths of the chemical were used to kill the eggs.

In this experiment a dam situated at Tengeru, 10 miles (16 km) from Arusha in Northern Tanzania, was used which has a small water inflow from 2 inlet furrows and a natural spring. The furrows were apparently snail-free and so eradication would be expected if a 100% kill of snails and eggs could be obtained. With relatively static water 2 treatments 14 days apart were decided upon, using the second method in an eradication attempt.

METHODS

Measurement of water volume

The volume of water to be treated was calculated from detailed measurements of the water surface

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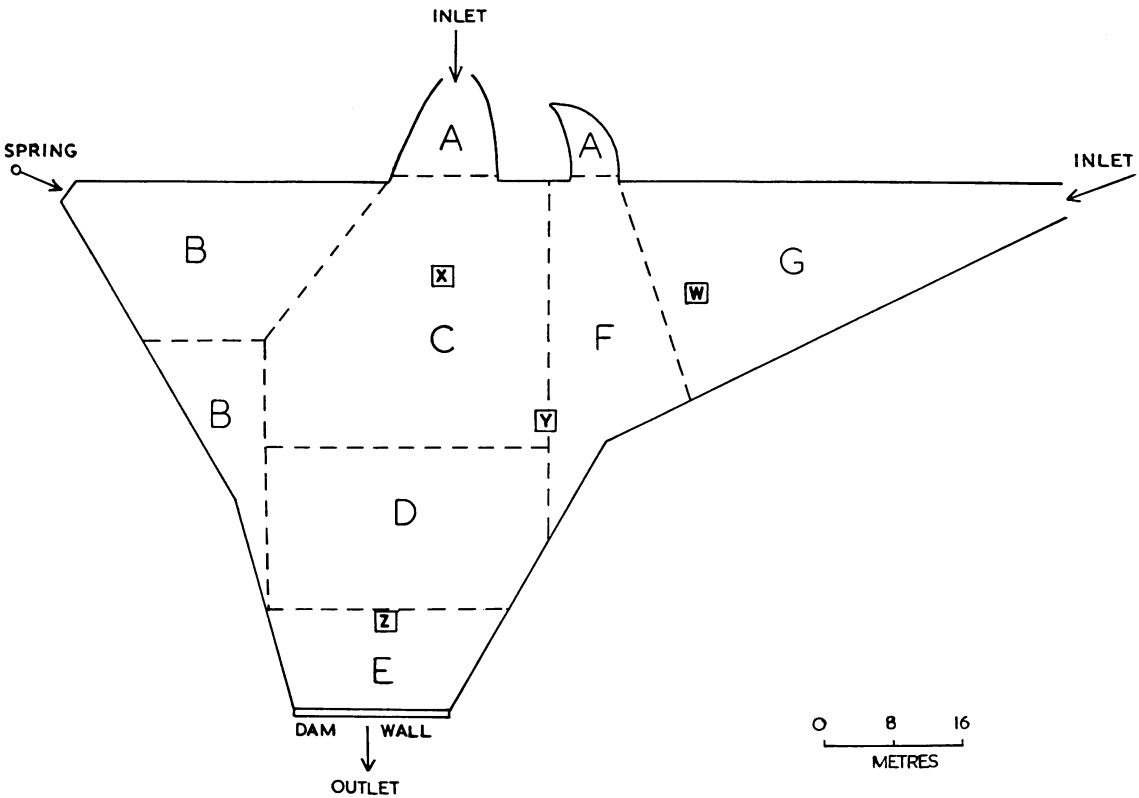
FIG. 1
PLAN OF TENGERU DAM



area and depth readings. A plan of the area was drawn and on it the pond was divided into blocks of approximately equal depth (Fig. 1). The volume of water in each section was calculated, and the sum of these volumes gave the total volume.

Treatment

In all, 5 applications of *N*-tritylmorpholine were made, and in each the same quantity of active ingredient was used—sufficient to give an over-all concentration of 0.25 ppm. The first application was used to assess the effect of the chosen dosage rate on the adult snails, and when a reasonable kill was obtained, the same dosage was applied twice with a 14-days interval in an eradication attempt. The same application methods and the same molluscicide formulation were used in these 3 treatments, as follows.

A 16% emulsifiable concentrate formulation of *N*-tritylmorpholine (FX 28) was used, and the volume of chemical required to give 0.25 ppm in each of the sections shown in Fig. 1 was first calculated (Table 1). The chemical was then diluted with water in a knapsack sprayer and applied to the surface of the respective sections from a boat rowed back and forth across the area.

The edges of the dam were sprayed from the bank to give the same concentration of 0.25 ppm while the 2 inlet furrows and the spring were given a 2-hour continuous treatment with FX 28 sprayed into the flowing water at approximately 2 ppm. The stronger dose was applied to compensate for the shorter contact period as the applied molluscicide was quickly washed into the main body of the dam by the untreated water flowing in.

The reappearance of young snails in the region of the inlet furrows prompted a change in application technique for the second split-dosage eradication attempt. A small amount of *N*-tritylmorpholine, this time in the form of a 50% wettable paste

TABLE 1
WATER VOLUME IN THE TENGERU DAM AND THE VOLUME OF FX 28 REQUIRED TO TREAT AT 0.25 ppm ACTIVE INGREDIENT

Block (see Fig. 1)	Mean depth (m)	Volume (litres × 10^6)	FX 28 required (ml)
A	0.3	0.08	120
B	1.0	0.52	780
C	1.3	0.84	1 250
D	2.0	0.84	1 250
E	2.3	0.48	710
F	1.7	0.44	675
G	1.7	0.85	1 275
Edge	0.3	0.16	250
Total		4.21	6 310

(FX 1300), was applied to the edges of the dam, but the remainder was divided into 9 parts. On 3 consecutive days 1 of the 9 portions was applied over a period of 3 hours to the spring and to each of the 2 inlet furrows. It is estimated that the concentration of chemical added to the flowing water during the hours of application was approximately 6 ppm. No chemical was sprayed on the surface of the main waterbody and the dispersion of *N*-tritylmorpholine from the 3 inlets (the 2 inlet furrows and the spring) was relied upon to reach all areas of the dam. The second application was added on days 15, 16 and 17 from the start of the first.

Snail population studies

Using the mud-sampling technique described by Crossland (1962), live and dead *B. pfeifferi* and *Lymnaea natalensis* counts were made daily so that weekly totals were directly comparable. The perimeter of the pond was sampled by taking 3 mud plugs from one point, combining the 3 samples, washing in a sieve and recording the numbers of live and dead snails. This procedure was then repeated at 5-yd (4.5-m) intervals around the dam. All snails were returned to the water after counting. The shoreline was sampled in this fashion on days 1 and 2 of a 6-day working week and then again on

FIG. 2
WEEKLY SNAIL COUNTS AT TENGERU DAM

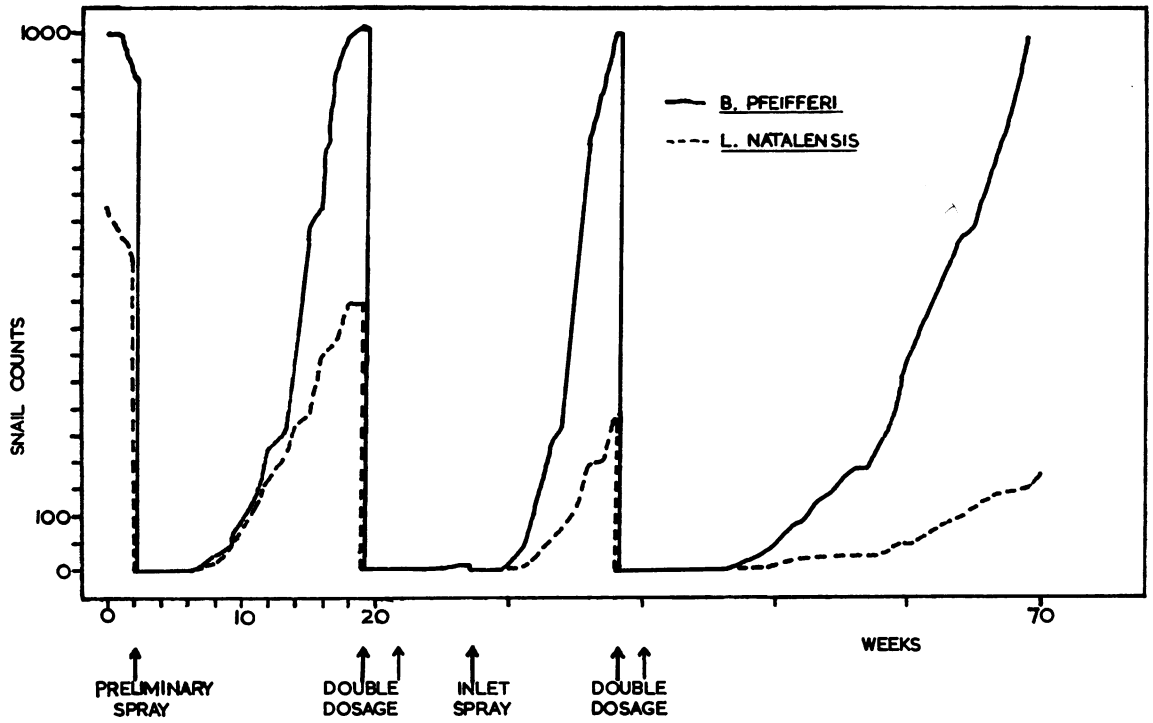


TABLE 2

WEEKLY SNAIL COLLECTIONS FROM TENGERU DAM

Week ending	Week No.	Live snails			Dead snails		
		B. pfeifferi	*L. natalensis*	Total	*B. pfeifferi*	*L. natalensis*	Total
5 Aug. 67	1	734	500	1 234	616	406	1 022
12 Aug. 67	2	809	545	1 354	606	502	1 108
19 Sept. 67	3	971	535	1 506	655	525	1 180
26 Sept. 67	4	1 004	673	1 677	703	594	1 297
2 Sept. 67	5	1 008	620	1 628	610	490	1 100
9 Sept. 67	6	918	511	1 429	604	479	1 083
11 September 1967: Preliminary treatment							
16 Sept. 67	1	0	0	0	1 902	1 524	3 426
23 Sept. 67	2	0	0	0	1 492	1 118	2 610
30 Sept. 67	3	0	0	0	1 218	871	2 089
7 Oct. 67	4	6	7	13	1 194	889	2 083
14 Oct. 67	5	24	13	37	1 055	818	1 873
21 Oct. 67	6	37	38	75	1 194	813	2 007
28 Oct. 67	7	80	80	160	995	774	1 739
4 Nov. 67	8	127	133	260	943	557	1 500
11 Nov. 67	9	223	188	411	928	576	1 504
18 Nov. 67	10	248	210	458	690	491	1 181
25 Nov. 67	11	349	263	612	850	540	1 390
2 Dec. 67	12	640	287	927	626	415	1 041
9 Dec. 67	13	677	406	1 083	517	394	911
16 Dec. 67	14	930	421	1 351	490	381	871
23 Dec. 67	15	1 065	501	1 566	515	426	941
6 Jan. 68	17	1 190	480	1 670	471	385	856
8 January 1968: First split-dosage treatment							
13 Jan. 68	1	0	0	0	2 013	1 137	3 150
20 Jan. 68	2	0	0	0	1 410	785	2 195
22 January 1968: Second split-dosage treatment							
27 Jan. 68	3	0	0	0	727	390	1 117
3 Feb. 68	4	0	0	0	485	310	795
10 Feb. 68	5	0	0	0	444	264	708
17 Feb. 68	6	0	0	0	382	247	629
24 Feb. 68	7	7	2	9	323	190	513
(28 Feb. 68; Inlet furrow sprayed)							
2 March 68	8	7	3	10	428	262	690
9 March 68	9	0	0	0	320	178	498
16 March 68	10	0	0	0	219	105	324
23 March 68	11	10	2	12	214	122	336
30 March 68	12	42	3	45	231	109	340
6 April 68	13	118	25	143	198	98	296

TABLE 2 (*concluded*)

Week ending	Week No.	Live snails			Dead snails		
		B. pfeifferi	*L. natalensis*	Total	*B. pfeifferi*	*L. natalensis*	Total
22 January 1968: Second split-dosage treatment (*concluded*)							
13 April 68	14	228	45	273	188	95	283
20 April 68	15	270	72	342	162	96	256
27 April 68	16	564	114	678	222	138	360
4 May 68	17	807	190	997	326	200	526
11 May 68	18	894	198	1 092	354	228	582
18 May 68	19	1 020	270	1 290	360	230	590
16, 17 & 18 May 1968: First 3-day treatment							
26 May 68	1	0	0	0	1 907	873	2 780
30 & 31 May and 1 June 1968: Second 3-day treatment							
2 June 68	1	0	0	0	1 652	594	2 246
9 June 68	2	0	0	0	1 314	492	1 806
16 June 68	3	0	0	0	1 195	441	1 636
23 June 68	4	0	0	0	763	341	1 104
30 June 68	5	0	0	0	596	271	867
7 July 68	6	0	0	0	430	213	643
14 July 68	7	0	0	0	374	187	561
21 July 68	8	3	0	3	273	146	419
28 July 68	9	16	0	16	206	113	319
4 Aug. 68	10	26	0	26	162	93	255
11 Aug. 68	11	52	4	56	169	97	266
18 Aug. 68	12	76	13	89	164	65	229
25 Aug. 68	13	91	15	106	176	62	238
1 Sept. 68	14	123	18	141	195	66	261
8 Sept. 68	15	141	18	159	173	56	229
15 Sept. 68	16	171	22	193	169	53	222
22 Sept. 68	17	190	18	208	188	73	261
29 Sept. 68	18	194	20	214	192	55	247
6 Oct. 68	19	242	27	269	184	59	243
13 Oct. 68	20	296	30	326	211	62	273
20 Oct. 68	21	384	49	433	204	63	267
27 Oct. 68	22	450	53	503	195	66	261
3 Nov. 68	23	495	61	556	218	72	290
10 Nov. 68	24	551	71	622	213	78	291
17 Nov. 68	25	635	94	729	249	92	341
24 Nov. 68	26	642	109	751	261	89	350
1 Dec. 68	27	719	119	838	302	113	415
8 Dec. 68	28	785	143	928	299	124	423
15 Dec. 68	29	854	141	995	286	129	415
22 Dec. 68	30	1 017	146	1 163	312	112	424
29 Dec. 68	31	1 130	142	1 272	292	124	416

days 4 and 5. Off-shore sampling from a boat was carried out on days 3 and 6 by taking 3 samples from 130 random positions on each day. Again the 3 samples were combined, washed and sieved and the snails counted. Sampling began 6 weeks before the preliminary application and continued until complete repopulation had taken place after the second eradication attempt. The weekly totals are tabulated in Table 2 and illustrated in Fig. 2.

Caged snails

Two days before every treatment, 4 cages each containing 25 *B. pfeifferi* were sunk at points in the centre of the dam. These were checked 48 hours after the molluscicide application and then at 24-hour intervals for 1 week or until all the snails died.

This served to check that the molluscicide reached the deepest parts of the pond in lethal concentrations.

Chemical analysis

Water samples (250 ml) were collected at points W, X, Y and Z (Fig. 1) 15 cm and 1.5 m below the surface, and transported to the laboratory in glass bottles for analysis. The samples were taken before and 24 hours after the preliminary spray (from positions X (15 cm and 1 m) and Z only); before and then 2 hours, 20 hours, 70 hours and 168 hours after each of the first eradication attempt applications; and finally before and on the third day of the second eradication attempt applications.

For the analysis a slight modification of the method of Crossland et al.[1] was used. The *N*-tritylmorpholine in solution was extracted with isohexane and the organic layer was then shaken with 98% sulfuric acid. The intensity of the resulting yellow solution was measured on a spectrophotometer and the concentration of *N*-tritylmorpholine (in ppm) read off from a standard graph.

A pH reading was taken on all pretreatment samples collected on 8 and 22 January 1968.

RESULTS AND DISCUSSION

Preliminary treatment

With the volume of the dam being calculated at approximately 4.21 million litres, the volume of FX 28 required to give an over-all concentration of 0.25 ppm was 6.3 litres (Table 1).

The *N*-tritylmorpholine sprayed on to the water surface sank slowly as a white layer. Dispersion was helped to some extent by the turbulence from the rowing boat and chemical analysis indicated that the molluscicide had penetrated satisfactorily. At 24 hours after the treatment the results were 0.20 ppm and 0.23 ppm *N*-tritylmorpholine at 15 cm, 0.35 ppm at a depth of 1 m (position X) and 0.34 ppm at a depth of 1.5 m (position Z).

Snail population studies yielded 1000 live *B. pfeifferi* and 700 live *Lymnaea natalensis* in the week before treatment and no live snails at all during the first 3 weeks after treatment. A few young snails were found during the fourth and fifth weeks and then the numbers built up rapidly, reaching the pretreatment level of 1000 *Biomphalaria* per week after 13 weeks.

The first split-dosage treatment

From the first day of this treatment (8 January 1968) no snails at all were found in the dam until the eighth week (the sixth week after the second spray). Young individuals appeared in the area of the 2 inlet furrows and a separate spray was applied immediately to kill them. More young snails were found during the twelfth week and by the nineteenth week the whole dam was repopulated to prespray levels.

Chemical analysis showed that the *N*-tritylmorpholine was satisfactorily dispersed (Table 3). Two hours after spray 1 the molluscicide was concentrated near the surface (e.g., for position W 0.75 ppm at 15 cm and 0.078 ppm at 1.5 m) but by the following day there was an almost uniform 0.2-ppm concentration at all sampled positions. At 72 hours after spraying the concentration was 0.03 ppm but this was still above the level required to obtain a 100% kill in the laboratory (0.025 ppm).

The pH of the lake water was 7.8 $\pm$ 0.2 for the 8 pretreatment samples taken before each of the two sprays.

Caged snails on the pond bed were all killed within 72 hours, but 94% had died after only 48 hours. The 6 survivors were in the cage placed at the deepest point in the pond (2.3 m at position Z).

After each spray many fish swam unsteadily to the surface and many died. Even after the second application, however, fish up to 30 cm in length were seen alive.

The second split-dosage treatment

The dam remained snail-free until the ninth week from the start of this double treatment and then

[1] Crossland, N. O., Webley, D. J. & Mesmer, E. T. (1964) *Miscellaneous report No. 433*, Arusha, Tropical Pesticides Research Institute (unpublished).

TABLE 3
CHEMICAL ESTIMATIONS OF *N*-TRITYLMORPHOLINE (ppm) TAKEN DURING THE FIRST ERADICATION ATTEMPT

Time of sampling	Position W		Position X		Position Y		Position Z	
	15 cm	1.5 m	15 cm	1.5 m	15 cm	1.5 m	15 cm	1.5 m
Before spray 1	0	0	0	0	0	0	0	0
After spray 1:								
2 h	0.75	0.078	0.54	0.07	0.54	0.028	1.040	0.066
20 h	0.23	0.175	0.175	0.210	0.246	0.165	0.195	0.206
70 h	—	0.075	0.025	0.025	0.045	0.020	0.035	0.019
168 h	0	0	0	0	0	0	0	0
Before spray 2	0	0	0	0	0	0	0	0
After spray 2:								
2 h	0.47	0.29	0.53	0.03	0.15	0.135	0.35	0.05
20 h	0.08	0.12	0.09	0.14	0.10	0.14	0.09	0.09
70 h	0.04	0.04	0.05	0.05	0.04	0.05	0.05	0.03
168 h	0	0	0	0	0	0	0	0

young *B. pfeifferi* again appeared in the region of the inlet furrows, while *Lymnaea natalensis* were not seen until the twelfth week. The build-up to former levels was slower than in the previous trial, taking 31 weeks to reach pretreatment levels. This may have been due, however, to a seasonal effect as there was a very fast population increase in the warmer months of January and February.

Chemical analysis showed that the molluscicide dispersed satisfactorily despite its being added only to the inlet furrows and to the dam perimeter. The reason for the high concentration of *N*-tritylmorpholine in position X (Table 4) is that it was in direct line with one of the inlets receiving a 6.0-ppm treatment.

Despite the care taken to reach every living snail in the waterbody, snails of both species returned to the dam. The first young specimens appeared at the same places around the inlet furrows during the ninth week. The conclusion must be either that the procedure failed to kill the necessary 100% of snails, eggs or both, or, possibly, that a few snails were present upstream and by the ninth week one or two had been washed in and began repopulation. Extensive searching has so far failed to reveal any such pocket of snails, so it would appear that while a very high snail kill is possible, eradication is just out of reach with present techniques. If this is so, single treatments at 5-week intervals probably represent the best means of control with *N*-tritylmorpholine.

TABLE 4
CHEMICAL ESTIMATIONS OF *N*-TRITYLMORPHOLINE (ppm) IN WATER SAMPLES TAKEN DURING THE SECOND ERADICATION ATTEMPT

Time of sampling	Position W		Position X		Position Y		Position Z	
	15 cm	1.5 m	15 cm	1.5 m	15 cm	1.5 m	15 cm	1.5 m
24 h after treatment 1	0.058	0.061	0.360	0.090	0.100	0.071	0.061	0.049
24 h after treatment 2	0.058	0.085	0.480	0.110	0.087	0.057	0.050	0.087

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RÉSUMÉ

TENTATIVES EN VUE D'ÉLIMINER LES MOLLUSQUES D'UN RÉSERVOIR D'EAU PAR L'EMPLOI DE LA *N*-TRITYLMORPHOLINE

Après un essai préliminaire visant à déterminer les doses actives du molluscicide, on a procédé à deux tentatives d'éradication de *Biomphalaria pfeifferi* et de *Lymnaea natalensis* d'un réservoir d'eau, d'une capacité de 4,5 millions de litres, situé près d'Arusha (Tanzanie).

Au cours de la première tentative, un concentré pour émulsion de *N*-tritylmorpholine a été pulvérisé sur la surface de l'eau de manière à obtenir une concentration de 0,25 partie par million. Le traitement a été renouvelé à 14 jours d'intervalle. La réapparition de jeunes mollusques dans le système d'alimentation du réservoir a contraint à recourir à une autre technique. Pour le second essai, la *N*-tritylmorpholine, sous la forme d'une pâte à 50% dispersable dans l'eau, a été introduite dans le réseau d'alimentation pendant 3 heures durant 3 jours consécutifs, le traitement étant répété après 2 semaines.

Dans chaque cas, les analyses chimiques d'échantillons d'eau ont montré une dispersion satisfaisante du molluscicide, et des mollusques encagés, immergés en divers endroits au centre du bassin, ont présenté une mortalité de 100% dans les 72 heures suivant l'application.

Après l'essai préliminaire comportant un traitement unique, les mollusques ont fait leur réapparition dès la 4e semaine. A la suite des deux traitements doubles, le réservoir est resté libre de mollusques pendant 8 et 9 semaines respectivement. Dans chaque cas, les premiers signes de repopulation ont été observés à proximité des canaux d'admission d'eau. Deux hypothèses sont avancées pour expliquer l'échec de ces diverses tentatives: la recolonisation du réservoir est due à l'introduction dans le milieu de mollusques en provenance de l'amont du réseau d'alimentation ou bien la quantité de *N*-tritylmorpholine employée n'a pas été suffisante pour détruire tous les mollusques et leurs oeufs avant que le courant ne la disperse. En dépit de recherches intensives, aucun vecteur n'a été découvert en amont du réservoir.

Les auteurs concluent que si la *N*-tritylmorpholine entraîne une très forte mortalité chez les mollusques, elle ne permet pas d'obtenir l'éradication avec les techniques actuelles. Des traitements uniques, renouvelés à intervalles de 5 semaines, paraissent devoir donner les résultats les plus favorables.

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