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POTENTIALLY HAZARDOUS SUBSTANCES IN SURFACE WATERS

II. CHOLINESTERASE INHIBITORS IN DUTCH SURFACE WATERS*

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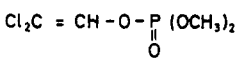
ABSTRACT

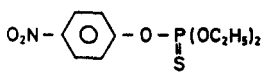
Several analytical methods were employed to determine the concentrations of cholinesterase inhibitors in several Dutch surface waters. An Auto-Analyzer method was used for screening purposes; thin-layer chromatography and gas-liquid chromatography–mass spectrometry were used for identification and quantitation of malathion, parathion, dimethoate, diazinon and carbaryl in extracts of the river Rhine.

INTRODUCTION

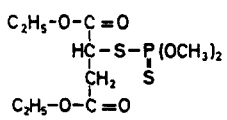
Cholinesterase inhibitors are used on a large scale as insecticides. Among them are esters of phosphoric acid (*e.g.* dichlorvos), of thiophosphoric acid (*e.g.* parathion), of dithiophosphoric acid (*e.g.* malathion) and carbamates (*e.g.* carbaryl).



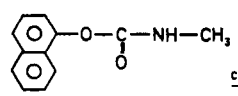
dichlorvos



parathion



malathion



carbaryl

* For part I, see ref. 1.

Contamination of surface waters by cholinesterase inhibitors can be of agricultural, as well as industrial origin. At the time this investigation started, no information was available on the occurrence of cholinesterase inhibitors in Dutch surface waters. Initially, the samples were screened on the basis of total cholinesterase inhibition, followed by identification and quantitation of the individual components. Information was also required on the behaviour of cholinesterase inhibitors during the preparation of drinking water.

The sampling plans were established in cooperation with the Head Inspection for Environmental Hygiene of the Dutch Department of Public Health. The survey program started in May 1969, and is still under way. The results reported in this paper cover the period May 1969–December 1971.

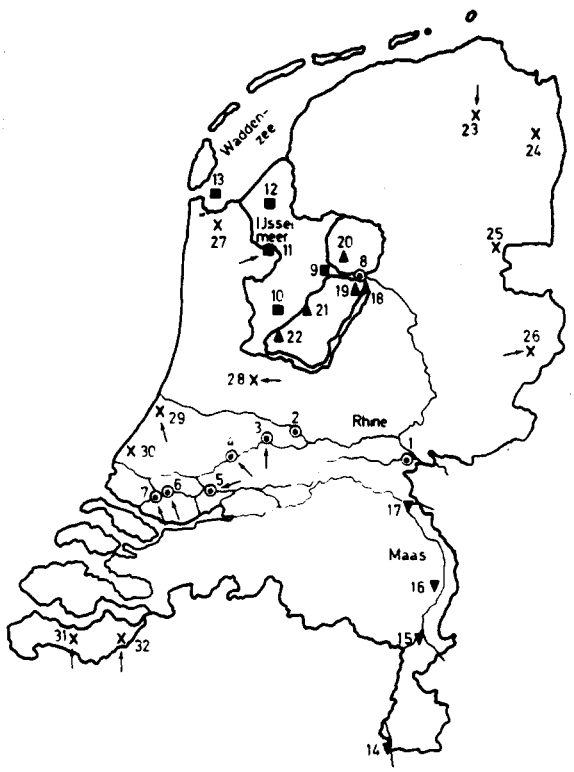


Fig. 1. Sampling sites (see also Tables I and II). ⊙, sampling sites 1–8, region Rhine and tributaries; ■, sampling sites 9–13, region IJsselmeer/Waddenzee; ▼, sampling sites 14–17, region Maas and tributaries; ▲, sampling sites 18–22, region IJsselmeer-polders; ×, sampling sites 23–32, other surface waters. The arrows indicate the sites where surface water is used as a starting material for the preparation of drinking water.

EXPERIMENTAL

Sampling sites

The locations of the sampling sites are given in Fig. 1. At 12 of these sites (indicated by an arrow) surface water is used for the preparation of drinking water

for about one third of the Dutch population. A brief topographical description of the sampling sites is given in Table I.

Methods

The cholinesterase inhibitors were determined by: (*a*) a thin-layer chromatographic (TLC) method; (*b*) an enzymatic–colorimetric method [this determination is currently carried out on an Auto-Analyzer (A-A)]; and (*c*) a gas–liquid chromatographic (GLC) method with mass spectrometric (MS) detection.

Details on the first two methods have been described earlier[1]. Details of the GLC–MS procedure are described below. The TLC and A-A techniques complement each other: the former gives information on the number of cholinesterase inhibitors present, whereas the latter gives information on the total cholinesterase-inhibiting capacity of the sample. With the A-A technique alone, identification of the cholinesterase inhibitors is not possible. With the TLC technique, indications can be obtained for the identification; however, further investigation by GLC and MS is necessary for confirmation.

The TLC and A-A methods are rapid, relatively inexpensive screening methods; GLC is the method of choice for the quantitative determination of specific compounds.

The detection limit of the TLC method is (depending on the pesticide involved) 0.01–0.05 ppb; with the A-A method 0.02 ppb (as paraoxon) is still determinable; the GLC–MS procedure allows quantitative determination of 0.01–0.05 ppb depending on the pesticide involved.

Unless stated otherwise, the results reported below pertain to methylene chloride extracts of surface waters inclusive of silt.

RESULTS

The investigations reported here can be divided into four parts: (*a*) screening of surface waters throughout the country; (*b*) the concentration of the cholinesterase inhibitors in different stages of the processing of drinking water; (*c*) more detailed investigations at sampling site 1 (see Fig. 1); and (*d*) identification and quantitative determination of some cholinesterase inhibitors.

(*a*) *Screening of different Dutch surface waters*

Samples of surface waters throughout the country were screened by the A-A technique. The screening involved drinking water reservoirs as well as surface waters along industrial, agricultural and horticultural areas. In the course of the investigagation, the sampling sites were changed following the needs and possibilities.

The results are summarized in Table II (for sampling site 1 the monthly averages are given, the other values correspond to haplo samples). As a further illustration the averages over the year 1970 are represented in Fig. 2.

(*b*) *Behaviour during the processing of drinking water*

The figures mentioned in Table II pertain to crude surface water samples. Apart from these, a limited number of samples were investigated in the same way at

TABLE I

DESCRIPTION OF THE SAMPLING SITES AND CORRESPONDING SURFACE WATERS (*cf.* Fig. 1)

Sampling site	*Region*	*Name of the surface water*	*Place*	*Remarks*
1	Rhine and tributaries	Waal	before Nijmegen at Rhine kilometre 883	Main branch of the Rhine river
2		Kromme Rijn	Beverweert	Relatively small, canalized branch of the Rhine
3		Amsterdam-Rijn canal	Jutfaas	Pumping stations for transport of surface water to the dunes along the seashore, where the water is infiltrated. The infiltrated water is used *i.a.* by the drinking water works of The Hague and Amsterdam
4		Lek	Bergambacht	
5		Wantij	Dordrecht	Drinking water station
6		Oude Maas	Berenplaat	Drinking water station for Rotterdam
7		Spui	Oud-Beijerland	Drinking water station
8		IJssel	Ketelhaven	Sample taken in the mouth of the river
9	IJsselmeer/Waddenzee	Ketelmeer	Ramspol	
10		IJsselmeer	off Lelystad	
11		IJsselmeer	Andijk	Drinking water station
12		IJsselmeer	off Stavoren	
13		Malzwin	N.E. of Den Helder	Part of the Waddenzee. A large amount of water transported by the Rhine enters the Waddenzee through this sea-arm.

14	Maas and tributaries	Maas	Eijsden	
15		Roer	Roermond	Sample taken in the mouth of the river
16		Rijnbeek	Venlo	Agricultural district
17		Niers	Gennep	Sample taken in the mouth of the river
18	IJsselmeerpolders	Hoge Vaart	Colijn	Large agricultural area. Treatment with pesticides is regularly carried out by spraying from the air. The samples are taken in the neighbourhood of polder pumping stations
19		Lage Vaart	Colijn	
20		(polderditch)	Banterweg	
21		Lage Vaart	Wortman	
22		Lage Vaart	De Blocq van Kuffeler	
23	Other surface waters	Drentse A	De Punt	Drinking water station
24		Ruiten A	Vlagtwedde	Agricultural district
25		Overijsselse Vecht	Haandrik	Agricultural district
26		Twente canal	Enschede	Drinking water station
27		Oude Veer	Anna Paulowna-polder	Bulb-growing district
28		Loenerveense plas	Loenerveen	Drinking water station
29		Valkenburgse Watering	Katwijk	Drinking water station. Water from the Kromme Rijn can come into this water system
30		Booma Wetering	Loosduinen	Horticultural district
31		Braakman	Braakman	Drinking water station
32		Kom West	St. Jansteen	Drinking water station

TABLE II

CHOLINESTERASE INHIBITORS IN DUTCH SURFACE WATERS (AUTO-ANALYZER TECHNIQUE)

Values in paraoxon-equivalents (ND = not detectable, *i.e.* <0.02 paraoxon-equivalents)

Sampling site[a]	*Region*	*Year*	*1969*								*1970*			
		Month	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*	*J*	*F*	*M*	*A*
1	Rhine and tributaries						2.74	4.40	5.83	2.51	0.89	0.49	0.71	0.66
2			0.83	0.62	0.71	1.56	1.17	2.04						
→ 3				0.70			1.42	1.20					0.22	
→ 4				0.80			1.38	1.52					0.28	
→ 5			0.77					1.82						0.74
→ 6			0.78		1.28	0.60		3.02						0.27
→ 7														0.83
8														
9	IJsselmeer/ Waddenzee													0.63
10														
→ 11													0.27	
12														0.34
13														0.15
14	Maas and tributaries			0.44				0.20	0.23	0.15			0.20	
15														0.04
16														
17							0.19		0.16					0.05
18	IJsselmeer-polders													0.04
														0.05
19														
20														
21														0.12
22														0.11
→ 23	Other surface waters													
24														
25														
→ 26														
27														
→ 28														
→ 29						0.13		0.32						0.43
30				0.40			0.27	0.57						
→ 31														0.04
→ 32														ND

[a] At the sites denoted by an arrow, surface water is used as a starting material for the preparation of drinking water

								1971											
M	*J*	*J*	*A*	*S*	*O*	*N*	*D*	*J*	*F*	*M*	*A*	*M*	*J*	*J*	*A*	*S*	*O*	*N*	*D*
0.43	0.67	0.78	1.19	1.92	1.84	0.64	0.27	1.09	1.00	0.54	0.54	0.17	0.23	0.16	0.32	0.41	0.14	0.68	0.98
0.25	0.38	2.08		1.96		0.22	0.42												
		0.82				0.52													
		1.10				0.36													
			0.48	2.00		0.52													
	0.30						0.80												
			1.08				0.23												
											1.26	0.34	0.14	ND	0.32	0.22	0.26	0.42	0.63
				0.35						0.28		0.40	0.06	ND	0.26	0.18	0.17	0.20	0.22
0.21		0.24																	
0.04			0.06	0.04			0.13			0.14		0.14	0.15		0.06	0.22	0.07	ND	
0.25		0.19		0.12															
	0.06		0.03			0.10													
0.41		0.09		0.50	0.42	0.09	0.22		0.08	0.08	0.24	0.08	0.08	0.03	ND	0.04	0.25		ND
	0.07	0.05			0.05	0.12			0.12		0.12		ND		ND		0.08	0.09	
0.09	0.10	0.07	0.06		0.23	0.53													
	0.04	0.09	0.03		0.06	0.11			0.08		0.08		ND		0.04		0.08		
	0.04	0.09	0.03	0.24						ND	0.22	0.24	0.12		0.46		0.25		
	0.06	0.04	0.11	0.23						ND	0.40	0.08	0.04		0.10		0.14		
		0.14																	
	0.05	0.13	0.08	0.13						ND		0.32	0.18	ND	0.06		ND		
	0.05	0.07	ND	ND															
	0.06	0.06			0.18														
0.10			0.05	0.05	0.03														
0.09		0.07		0.02		ND													
0.03		0.06					0.07			ND		ND			ND		ND		ND
		0.10		0.05	0.33	0.15	0.05												
ND						0.02													
	0.16			0.30	0.50										0.08	0.35	0.44	1.18	
0.11	0.14	0.12	0.06		0.40														
	0.03					0.02													
	ND					0.03					ND	ND	0.04	ND	ND				

(*cf.* Fig. 1 and Table I).

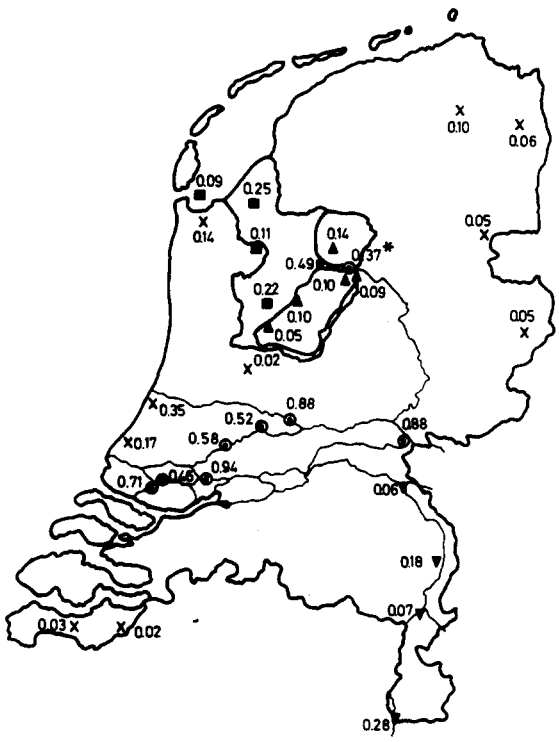


Fig. 2. Cholinesterase inhibitors in Dutch surface waters, Auto-Analyzer technique; averages over the year 1970 in paraoxon-equivalents.

different stages of the purification to drinking water. Although there were large differences in working conditions from one station to the other, and consequently large deviations between individual samples, a general trend can be found:

Treatment	*Elimination of cholinesterase inhibitors*
Rapid filtration	– average 0%
Slow sand filtration	5–50%; average 30%
Infiltration	30–50%; average 45%
$Fe(OH)_3$ flocculation	30–90%; average 60%

Unfortunately no information was available on the elimination by active carbon, as this method seems most promising[2]. Further investigation on the eliminating capacity of the different procedures used in the preparation of drinking water is necessary.

(*c*) *Investigations in the river Rhine*

From the results presented in Table II, it follows that the highest concentrations of cholinesterase inhibitors are found in the river Rhine and its tributaries. This river

was therefore studied in more detail: from September 1969 onwards, samples were taken three times a week at site 1 (Fig. 1) and examined not only for cholinesterase inhibitors, but also for organochlorines and endosulfan[1-5].

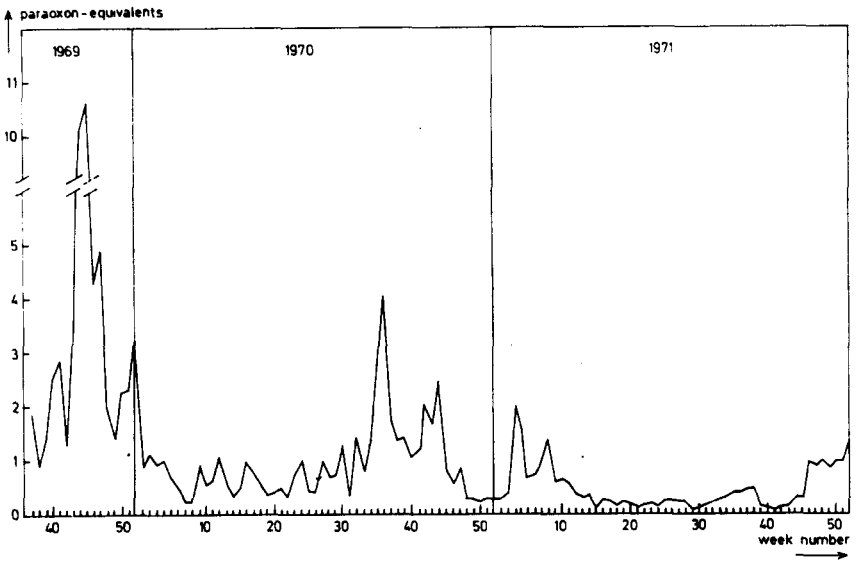


Fig. 3. Cholinesterase inhibitors at Rhine kilometre 883 (site 1 in Fig. 1), Auto-Analyzer technique; weekly averages in paraoxon-equivalents.

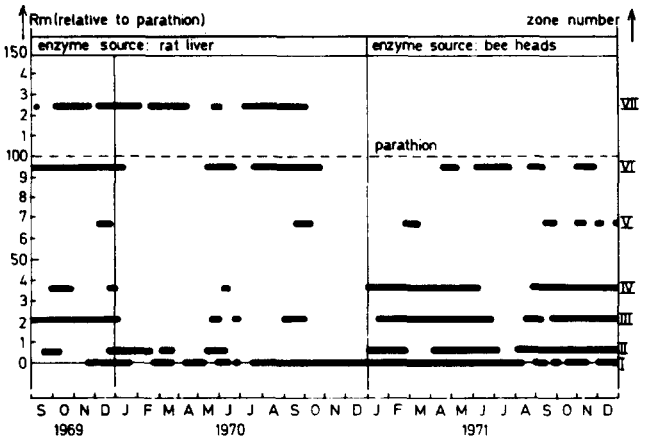


Fig. 4. Cholinesterase inhibitors at Rhine kilometre 883 (site 1 in Fig. 1), TLC technique.

The weekly averages of the cholinesterase-inhibiting capacity, as determined by the A-A technique, are illustrated in Fig. 3. The thin-layer chromatograms are summarized in Fig. 4. The R_F values of the spots have been standardized to R_m values relative to parathion (the average R_F value of parathion is 0.54, the front distance 14 cm).

The spots can be grouped in seven zones (numbered I to VII in Fig. 4), corresponding to the following R_m values (average value and 95% confidence limits given):

Zone number	R_m *value (parathion = 100)*
I	0
II	6±1
III	21±1
IV	36±1
V	67±1
VI	94±1
VII	124±2

From January 1, 1971 onwards bee heads were used as the enzyme source instead of rat liver. The bee head homogenate gives sharper spots and a better sensitivity in the lower region (zone numbers II–IV) of the chromatograms.

The R_m values of the standard substances used (average value, 95% confidence limits and amount brought on the plate given) are:

Substance	R_m *value*	*Amount in standard solution (ng)*
Dimethoate	0	not used in standard
Carbaryl	7±1	0.5
Malathion	24±1	1.0
Diazinon	37±2	2.0
Fosalone	59±1	1.0
Parathion	100	1.0
Dichlofenthion	124±2	2.5
Carbofenothion	136±1	2.5

Apart from the chromatograms summarized in Fig. 4, for which benzene was used as the solvent, parallel chromatograms were prepared from January 1, 1971 onwards with benzene–acetone–*n*-hexane (10:25:65) as solvent; the oxidation step was left out in order to detect more specifically the carbamates and organo-phosphates. All samples investigated showed strong inhibition spots at R_m 99 ±1 relative to carbaryl (R_F of carbaryl = 0.29).

(d) Identification and quantitative determination

Identification of some frequently occurring inhibitors was carried out by means of MS. Prior to this the samples were cleaned by preparative TLC. Two different solvents were used in every clean-up in order to increase the specificity.

The wide use of dimethoate, carbaryl, malathion, diazinon and parathion and the results of the TLC suggested the presence of these insecticides. The MS identification was therefore focussed on these five insecticides.

The clean-up of zones I and II (supposedly containing dimethoate and carbaryl) was carried out on preparative thin-layer plates (1 mm SiO_2) with benzene–acetone (95:5) and benzene–acetone–*n*-hexane (10:25:65) as solvents. The clean-up of zones III–VI (supposedly containing malathion, diazinon and parathion) was carried out in the same way with cyclohexane–*n*-hexane–acetone (50:40:10) and methylene-chloride–benzene (80:20) as solvents. When the identification work started, zone VII did not contain further detectable amounts of cholinesterase inhibitors (*cf.* Fig. 4).

The presence of the five insecticides was confirmed by MS (Varian Mat CH_5 instrument). The quantity of the phosphorus esters dimethoate, malathion, diazinon and parathion was then determined with a mass spectrometer–gas chromatograph combination. The mass spectrometer was used as a specific detector for the gas chromatograph. The mass spectrometer was fixed on a characteristic *m/e* value and mass fragmentography methods were used. If a mass fragmentogram is taken at only one *m/e* value the substance is characterized by its retention time and the occurrence on the characteristic *m/e* value. If this procedure is not reliable enough, other mass fragmentograms must be taken. In several cases this was carried out.

The gas chromatographic conditions were: column: Pyrex, 10 ft. long, 1/8 in. O.D., filled with OV-17 on Aeropak-30 (80–100 mesh), 200 °C; gas: He, 45 ml/min; retention times: dimethoate, 5 min 25 sec (characteristic *m/e* value, 229); malathion, 10 min 15 sec (*m/e* 173); diazinon, 4 min 12 sec (*m/e* 304); parathion, 10 min 20 sec (*m/e* 291).

The retention times of the peaks obtained in this way did not deviate more than ± 3 sec from the retention times obtained from standards (spiked samples).

Gas chromatography in combination with a phosphorus flame photometer was also used in the identification work. Although also highly specific, this detector proved to be more subject to interferences, and to be less sensitive than the MS detection.

Carbaryl, which is difficult to handle on the gas chromatograph, was quantitatively measured on the mass spectrometer. The two-fold cleaned extract was brought with the direct insertion probe into the ionization chamber. The sample was then heated according to a preset temperature program. The time dependence of the intensity of three characteristic ions ($m/e = 201.078979$, 144.057515 and 116.062600) was then recorded in high resolution. The recorded peaks appeared in

TABLE III

CONCENTRATION (ppb) OF CHOLINESTERASE INHIBITORS IN RHINE WATER

Cholinesterase inhibitor	*Sample A (November 1971)*	*Sample B (January 1972)*
Dimethoate	0.07	0.08
Malathion	0.01	0.01
Diazinon	0.02	0.05
Parathion	0.03	0.07
Carbaryl	0.40	0.20

the right intensity ratio, which indicated the presence of carbaryl. A comparison with a standard, treated in the same way, made a quantitative measurement possible.

The concentrations of the five identified cholinesterase inhibitors were determined in two typical samples of Rhine water. The results are given in Table III.

DISCUSSION

Several authors have described methods for the detection of cholinesterase inhibitors in surface waters: Askew *et al.*[6] described a TLC and a gas chromatographic method capable of detecting cholinesterase inhibitors down to a level of 1 and 0.1 ppb, respectively. Nicholson[7] proposed the brain-cholinesterase activity of fish, exposed to the surface water under investigation, as a parameter for the presence of cholinesterase inhibitors. Davies[8] and Colas[9] described the use of gas chromatography in combination with a thermionic detector (CsBr); the detection limit is in the order of 1 ppb. Weil and Quentin[10] proposed essentially the same method as described in this paper, not using the A-A, however. In these papers no data are given on the actual monitoring of samples, so that a comparison of results is not possible.

Lowden *et al*[11]. mention the presence of carbophenothion (0.01–1 ppb), diazinon (0.01–0.03 ppb), demeton or demeton-S (0.01 ppb), malathion (0.01 ppb) and phorate (0.01 ppb) in British surface waters, not giving details of the methods used. To our knowledge the most extensive reports on cholinesterase inhibitors in surface waters originate from Italy (Del Vecchio *et al.*[12] and Leoni and Puccetti[13]). These investigators used: (*a*) TLC with horse serum as the enzyme source and indoxylacetate as the substrate, or tetrabromophenol-phthaleine–ethyl ester and $AgNO_3$ as reagents; and (*b*) gas chromatography with electron capture or thermionic detection. Interestingly, the total cholinesterase-inhibiting capacity figures reported in this paper are of the same order of magnitude as those given for Italian surface waters, *viz.* 0.14–2.81 parathion-equivalents (as oxidation is carried out prior to analysis, parathion- and paraoxon-equivalents are identical in this case). The authors positively identified ronnel, parathion, methylparathion, malathion, dursban and diazinon. The concentrations reported a range from less than 0.01 ppb to about 0.70 ppb. Three of the six insecticides mentioned by the Italian authors were also found in Dutch surface waters, *viz.* parathion, malathion and diazinon. The presence of dimethoate was proven in Dutch surface waters and supposed in Italian surface waters.

It is conceivable that apart from the five cholinesterase inhibitors that have been identified, other cholinesterase inhibitors are present in the river Rhine. The screening and identification investigations will therefore be continued using *inter alia* different enzyme sources and different substrates.

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