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INSECTICIDES AS A FACTOR IN THE MORTALITY OF THE SANDWICH TERN (*STERNA SANDVICENSIS*). A PRELIMINARY COMMUNICATION

by

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Introduction

In 1965 it was reported (Koeman and van Genderen, 1965) that several sea bird species in the Dutch Wadden Sea contained residues of chlorinated hydrocarbon insecticides in their tissues in considerable amounts. One of these, the sandwich tern (fig. 1), showed a remarkable decline in the number of breeding pairs in the Netherlands during the laste decade (fig. 2). A toxicological study was made to determine the role of these insecticides in the decline. The study includes :

1. Chemical analyses of tissues, eggs and food.
2. Observations in the breeding colony at the island Griend in the Dutch Wadden Sea which is one of the few places in the Netherlands where the sandwich tern still breeds.
3. An analysis of the recovery data collected by the Bird Migration Station at Arnhem.
4. Pathological and parasitological examinations of the birds.
5. A distribution study to measure the extent of the coastal contamination by chemical analyses of indicator organisms such as mussels and birds' eggs.

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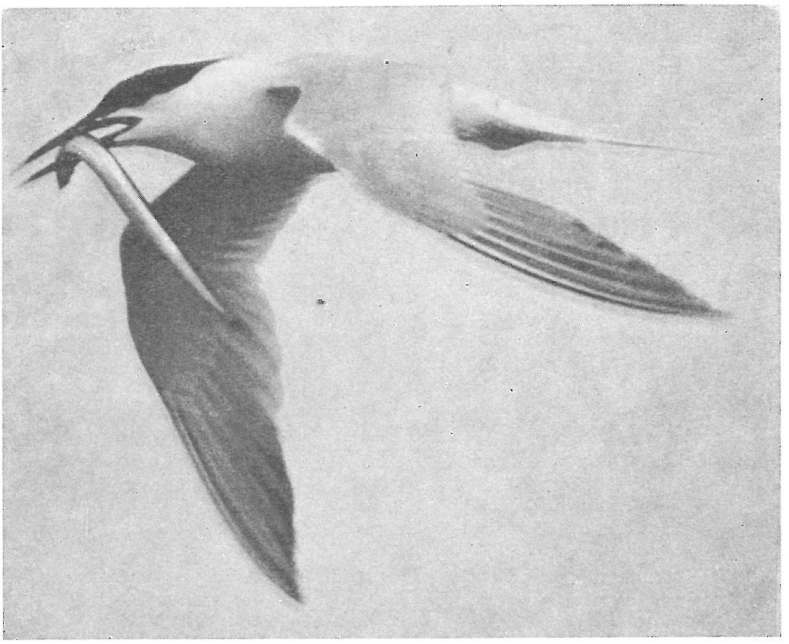

Photo J. Veen

Fig. 1. Sandwich Tern with Sand-eel

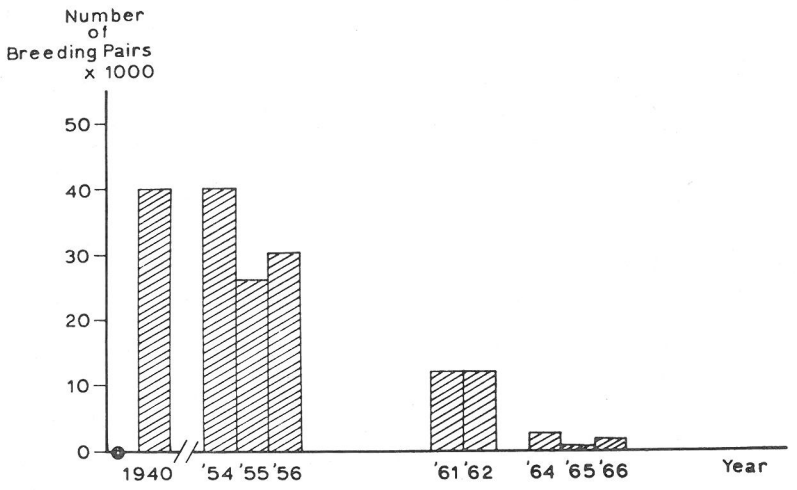

Fig. 2. Number of Breeding Pairs of the Sandwich Tern in the Netherland in the period from 1940 to 1966.

Methods

Chemical analyses were carried out as has been described previously. The following types of columns were used for gas liquid chromatography (GLC) :

1. DC 11 (5 %) on Chromosorb W 60/80 mesh.
2. SE 30 (5 %) + Epikote 1001 resin (0.3 %) on Chromosorb W 60/80 mesh.
3. Oronite 128 (3 %) + Epikote 1001 resin (0.3 %) on Chromosorb W 60/80 mesh. (Richardson, 1965)
4. A column packing consisting of intimately mixed equal portions of previously coated 80/100 mesh Gas Chrom Q; one portion with 15 % QF-1 and the other with 10 % DC 200 (Burke and Holswade, 1966)

For the thin layer chromatography (TLC) the modification of the spraying reagent as proposed by Abbott, Egan and Thomson (1964) was used. For identification also preparative TLC was used in conjunctions with GLC. The analytical procedures will be given in full detail elsewhere.

The pathological study consisted of a gross pathological examination of the dissected birds followed by a bacteriological examination of the liver and the heart with cultivation on serum agar plates. The intestinal contents were examined for the presence of *Salmonellae* by enrichment on Müller-tubes and subsequent inoculation on brilliant-green-phenol-red agar plates.

The parasitological examination consisted of a microscopic examination of the intestinal contents.

Results and discussion

Observations in the colony at the island of Griend in 1965.

A large number (about 10 % of the hatched birds) of the chicks died in June mostly at the age of one week or less. This in spite of the fact that the weather and the supply of fish were good. Many were seen dying in convulsion and the dead chicks were found in a characteristic posture with leggs stretched and a marked opisthotonus similar in appearance to white leghorn chicks poisoned experimentally with chlorinated hydrocarbon insecticides in the laboratory. Death occurred also among juvenile terns at the age of 3 to 6 weeks, the age at which they start flying (about 5 % of the hatched birds). Here also it was observed that birds died in convulsion. During the whole breeding season regularly adult birds were found dead in the colony.

It is interesting to note that even in this highly contaminated bird population only 10 % of the eggs failed to hatch, which is a normal figure.

Observation in the colony at the island of Griend in 1966.

In spite of careful observation of the colony by one of the authors (Veen), mortality of the characteristic kind observed in 1965 was not seen in 1966. A number of chicks died after a rainy and stormy period.

Chemicals analyses and interpretation of the residues found.

The following compounds could be identified in extracts from tissues and eggs.

1. Telodrin
2. Dieldrin
3. Endrin
4. pp' DDE and
5. Polychlorinated biphenyl compounds (PCB)

The PCB's were first identified by Jensen in Sweden (Anonymous, 1966) as responsible for unknown peaks with longer retention times than DDE in GLC.

The PCB peaks in the sandwich tern extracts have retention times similar to those of a solution of a PCB preparation containing 6 chlorine atoms per molecule (Phénoclor DP 6, kindly supplied to us by the « Société Progil « in Paris).

Table 1 shows the results of the analyses of the livers. For the PCB's only a qualitative indication is given. Quantitative analyses are in progress.

From the work of Keplinger and Deichmann (1965) we know that the toxic actions of dieldrin and endrin are additive (not strictly in a mathematical sense). From our work with hens' chicks concerning critical blood concentrations, the results of which will be published elsewhere, it was concluded that dieldrin and telodrin also have additive effects on the chicks. Six chicks, which hatched from eggs dosed with 960 µg of dieldrin before incubation and which died in convulsion about 3 to 4 days after hatching, had a mean liver residue of 90.4 ppm. Six other chicks which were dosed in the same way with 80 µg telodrin and 320 µg dieldrin died after 3 to 4 days after hatching with a mean liver residue of 3.1 ppm telodrin and 38.2 ppm dieldrin. From these figures combined with additive action it can be concluded that 1 ppm of telodrin is about equivalent to 17 ppm of dieldrin $\left(\frac{90.4 - 38.2}{3.1} = 17\right)$. If we calculate the data of the « drin « residues in table 1 into dieldrin values we find for the adults, the juveniles, and the chicks which were found

TABLE 1

Residues in the livers of sandwich terns found dead or killed in the Dutch Wadden Sea in the summers of 1965 and 1966

Age Class	Year	History	Number	Liver residue in ppm (wet weight) Geometric mean and range				
				Dieldrin	Telodrin	Endrin	DDE	PCB
Juvenile	1965	killed	3	0.31 (0.20-0.42)	0.07 (0.05-0.10)	0.12 (0.07-0.19)	0.14 (0.10-0.17)	+
Juvenile	1965	found dead or in convulsion	8	4.6 (1.9 -6.6)	0.86 (0.60-1.7)	0.43 (0.10-1.2)	1.70 (0.90-3.4)	+
Adult	1965	shot	5	0.84 (0.48-2.0)	0.23 (0.07-0.50)	0.29 (0.13-0.80)	0.58 (0.30-1.3)	+
Adult	1965	found dead	5	5.5 (4.7 -7.2)	1.0 (0.80-1.6)	0.67 (0.5 -0.8)	2.5 (1.9 -3.6)	+
Chick	1965	found dead or in convulsion	6	5.6 (2.4 -12)	2.3 (0.63-3.8)	0.47 (0.19-1.3)	5.9 (2.0 -12)	+
Chick	1966	found dead after a storm	6	0.63 (0.45-0.91)	0.14 (0.10-0.32)	0.42 (0.17-0.66)	1.2 (0.70-2.2)	+

dead or dying, in 1965 values of respectively 25, 20 and 45 ppm of dieldrin equivalents in the liver.

These values approximate the liver residues observed in birds and mammals which were poisoned experimentally with dieldrin (table 2). The details of these experiments will be published later. The result of an experiment of Turtle et al. is added in this table.

TABLE 2

Liver residues in animals which died after experimental poisoning with dieldrin

Species	Number	Liver residue in ppm (range)	Route of administration
Khaki Campbell Duck	5	31.0– 49.1	Tablet implantation
White Leghorn Chick	6	62.2–112	Injection into the yolk before incubation
Rat	10	7.8– 74.2	Tablet implantation
Feral Pigeon	4	15.0– 24.4	Repeated oral administration (Turtle et al., 1963)

The residues of DDE and the PCB's have not been included. DDE is much less toxic than the "drin"-compounds and from our preliminary results with egg toxicity trials it is concluded that the PCB's also are less toxic than dieldrin.

Further evidence for poisoning as the cause of death of the chicks of the sandwich tern can be derived from the egg toxicity trials with white leghorn chicks (Koeman, Oudejans and Huisman, 1967). In figure 3 it can be seen that the concentration of dieldrin in the blood of chicks from dieldrin treated eggs which were sacrificed at different intervals after hatching rises steeply during the first two days of life (which means an increased rate of exposure to the birds). This rise of the concentration in the blood corresponds with the absorption of the yolk which in birds continues after hatching. If the chicks start feeding the level declines. However if the food supply is suboptimal, the level continues to rise and eventually a lethal concentration is reached (in starved chicks, an average critical concentration in the blood was found of 11.5 µg/ml of dieldrin).

Pathological and parasitological examinations.

In the pathological examinations nothing was found, which could explain the death of the birds. No *Salmonellae* could be cultivated from the intestines.

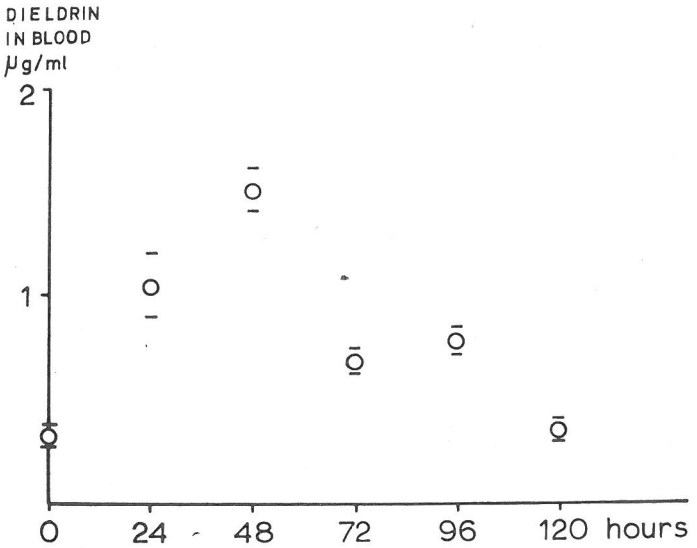

Fig. 3. Concentrations of dieldrin in the blood in normally fed hen's chicks which hatched from eggs treated with 240 µg of dieldrin before incubation. The chicks were killed at different times after hatching.

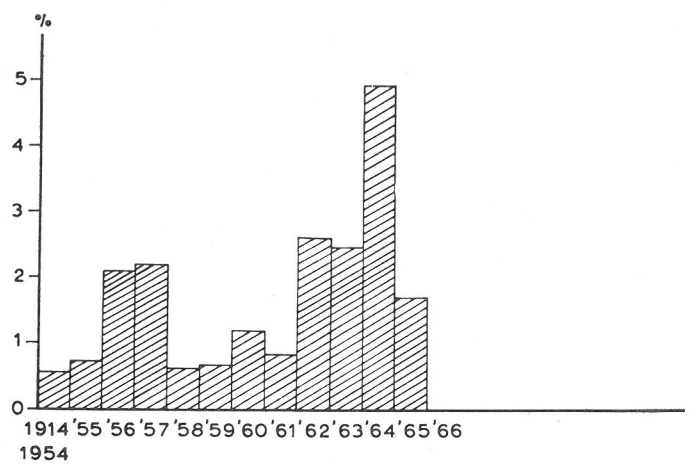

Fig. 4. Banded Juvenile Sandwich Terns found dead or dying expressed as percentage of the number of birds banded per year (birds found in the colony are not included).

Ten of the birds (juveniles and adults) have been examined on intestinal parasites in 1965. Only trematodes were found in three birds, a different species in each host. It does not seem likely that during the period of the investigation the breeding colony as a whole was suffering from a serious parasitic disease, for in this case one species would have been found in more hosts, and in larger numbers.

Analysis of the recovery data collected by the Bird Migration Station at Arnhem

Every year hundreds of sandwich tern chicks are banded in the colonies. The recovery data are collected by the Bird Migration Station at Arnhem.

In figure 3 the recoveries are given of sandwich terns which were found dead or dying outside the colony during their first year of life. The recoveries are given as percentages of the numbers banded each year.

From 1914 to 1954 5124 chicks have been banded, 29 of these were recorded dead or dying during their first year of life (0.56 %). When we compare the recovery percentages during the last decade with that of 1914 to 1954 it is evident that in several years an increased rate of mortality existed. This was particularly the case in 1964 when the first birds which were observed « to fall from the air » in convulsion reached our laboratory. At least for 1964 and 1965 the increased death rate can be correlated with a lethal contamination of the birds. At the moment of preparation of this paper no recoveries were reported of birds which were banded in 1966.

Distribution of chlorinated hydrocarbon insecticides in the coastal environment.

As the terns are specialized fish feeders (only very young chicks eat shrimp) the only way in which they can be contaminated by persistant chemicals is by the fish they eat.

Table 3 shows the residues in the fish species on which the terns feed, sprat *Clupea sprattus*, juvenile herring *Clupea harengus*, and sand-eel *Ammodytes lanceolatus* (fig. 1). Fish were collected in the Dutch Wadden Sea in 1965 and 1966. In the fishes the same compounds could be identified as in the birds. Particularly the dieldrin residues were much lower in 1966 than in 1965.

We further examined the distribution of the insecticides along the Dutch coast by using the mussel (*Mytilis edulis*) as an indicator organism (24 places were sampled in August 1965). Dieldrin and endrin were detected along the whole length of the coast. For telodrin relatively high figures (0.25 ppm) were found near the place where the water of the Rhine (« Nieuwe Waterweg ») enters the North Sea. Beyond this place the levels gradually declined.

TABLE 3

Residues in fish species on which the sandwich tern feeds (total body analysis)

Species	Date	Number	Residue in ppm (wet weight)				
			Geometric mean and range				
			Dieldrin	Telodrin	Endrin	DDE[1])	PCB
Sprat	summer 1965	43 (3 samples)	0.30 (0.16-0.42)	0.06 (0.06-0.07)	0.12 (0.07-0.22)	⩽ 0.14 (0.14-0.15)	+
Herring (juvenile)	summer 1965	38 (4 samples)	0.27 (0.26-0.29)	0.05 (0.04-0.06)	0.10 (0.07-0.20)	⩽ 0.11 (0.10-0.14)	+
Sand-eel	summer 1965	22 (4 samples)	0.25 (0.19-0.34)	0.04 (0.04-0.05)	0.21 (0.12-0.45)	⩽ 0.12 (0.11-0.15)	+
	total	103 fishes	0.27 (0.16-0.42)	0.05 (0.04-0.07)	0.14 (0.07-0.45)	⩽ 0.12 (0.10-0.15)	
Sprat	summer 1966	19 (10 samples)	0.10 (0.04-0.18)	0.02 (0.02-0.04)	0.11 (0.04-0.29)	⩽0.14 (0.05-0.23)	+
Sand-eel	summer 1966	18 (18 samples)	0.06 (0.01-0.23)	0.01 (0.01-0.05)	0.08 (0.01-0.26)	⩽ 0.08 (0.04-0.16)	+
	total	37 fishes	0.07 (0.01-0.23)	0.02 (0.01-0.05)	0.09 (0.01-0.29)	⩽ 0.10 (0.04-0.23)	

[1]) In most fishes DDE could not be measured accurately as a consequence of the presence of interferences.

TABLE 4

Residues in the eggs of several tern species from the Netherlands, Great Britain, Ireland and Germany

Region	Year	Number	Species[1])	Residue in ppm (wet weight)				
				Geometric mean range				
				Dieldrin	Telodrin	Endrin	DDE	PCB
Griend (N)	1965	8	ST	0.80 (0.36-2.4)	0.09 (0.02-0.45)	0.17 (0.10-0.34)	0.61 (0.29-1.2)	+
Griend (N)	1966	25	ST	0.23 (0.09-0.79)	0.06 (0.02-0.12)	0.20 (0.08-0.36)	0.61 (0.26-1.1)	+
Oldeoog (G)	1965	14	CT	0.70 (0.31-3.5)	0.08 (0.02-0.73)	0.24 (0.03-1.6)	0.54 (0.25-1.5)	+
Oldeoog (G)	1966	6	CT	0.29 (0.08-0.55)	0.06 (0.03-0.09)	0.08 (0.04-0.13)	0.44 (0.09-0.86)	+
Wexford (I)	1965	5	ST	0.12 (0.10-0.15)	n.d.[2]	n.d.[2]	0.64 (0.43-0.97)	+
Wexford (I)	1965	6	RT	0.05 (0.03-0.12)	n.d.[2]	n.d.[2]	0.24 (0.13-0.49)	+
Scolt Head (G.B.)	1966	5	ST	0.15 (0.08-0.41)	n.d.[2]	in one 0.03	0.49 (0.26-1.2)	+

[1]) With the abbreviations the following species are mentioned :
ST : Sandwich Tern (*Sterna sandvicensis*)
CT : Common Tern (*Sterna hirundo*)
RT : Roseate Tern (*Sterna dougalli*)

[2]) n.d. : not detectable

Since the first sampling took place in August 1965 every half year mussels are sampled from a few places along the coast between Hoek van Holland and Den Helder.

During 1966 we observed that telodrin disappeared from the mussels. In August 1965 a value of 0.11 ppm was found at Scheveningen and in July 1966 less than 0.003 ppm. No decline was observed for the contents of dieldrin and endrin in the mussels.

The coastal contamination in the Netherlands was compared with that in Ireland, Great Britain and Northern Germany by analysing tern eggs which were kindly supplied by colleagues from those countries. Table 4 gives the results of the analyses.

It can be seen that the occurrence of telodrin and endrin is restricted to the Dutch and adjacent German coast. The dieldrin residues are also higher in this area.

It is important to note that in the eggs (table 4) and in fish (table 3) the residues found in 1966 are much lower than in 1965. This decrease is correlated with the lesser mortality of the terns in 1966 and the absence of any evidence of death from convulsions in that year.

Very probably the contamination originates at least partly from the effluent of a pesticide producting factory near Rotterdam. The elaborate measures already taken by this firm to prevent further release of these insecticides as much as possible may have been the cause of the observed lesser residues.

The absence of visible mortality from poisoning in 1966 may indicate that the amounts of residue still present do not threaten the existence of the colony. Further work is planned to follow the residues in eggs as a measure of the hazard from poisoning and as indicator for the degree of contamination of the coastal environment in the Netherlands.

Conclusion

From the evidence presented it is concluded that in 1964 and 1965 mortality due to poisoning from the combined action of dieldrin, telodrin and endrin occurred in sandwich terns in the breeding colony at Griend.

It is difficult to say to what extent this mortality is responsible for the decline in the tern population noted in particular since 1962. The gerater part of the total mortality, of course, escapes observation. However, poisoning was mainly observed in young animals and the recovery data of bird-migration indicate a general increased mortality also in young animals. This similarity points

to the probability that death from poisoning occurred in a relatively large number of young terns.

The specialized feeding habit of the sandwich tern makes it particularly vulnerable to defects in its food. Such defects are likely to be detrimental at the population level.

As other deleterious factors of importance have not been found to act, we tentatively conclude that the population decline in the breeding colony is due to the contamination of the coastal environment with the chlorinated hydrocarbon insecticides mentioned. Further evidence since 1966 points to a decrease both of the environmental contamination at the coastal area and of poisoning in the tern colony. This may be the result of the precautionary measures which have been taken to limit the industrial contamination of the environment.

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LITERATURE

ABBOTT, D.C., EGAN, H. and THOMSON. J. : — Some observations on the thin-layer-chromatography of organo-chlorine pesticides. *J. Chromatogr.* **16** (1964) 481.

ANONYMOUS : — Report of a new chemical hazard. New Scientist, 15th December 1966, p. 612.

BURKE, J.A. and HOLSWADE, W. : — A gaschromatographic column for pesticide residue analysis : Retention times and response data. *Journal of the A.O.A.C.* **49**/2 (1966) 374.

KEPLINGER, M.L. and DEICHMANN, W.B. : — Toxicity of Combinations of Pesticides. *Toxicol. Appl. Pharmacol.* **7**, abstract 31 (1965) 487

KOEMAN, J.H. and van GENDEREN, H. : — Some preliminary notes on residues of chlorinated hydrocarbon insecticides in birds and mammals in the Netherlands. *Mededelingen van de Landbouwhogeschool en de Opzoekingsstations van de Staat* te Gent **30**/3 (1965) 1879.

KOEMAN, J. H., OUDEJANS, R. C. H. M. and HUISMAN, E. A. : — Danger of chlorinated hydrocarbon insecticides in birds' eggs. *Nature* **215** nr. 5105 (1967) 1094.

RICHARDSON. A, : — A new stationary phase for the separation of organochlorine insecticides. *Chem. Ind.* (London) 1965 (320) 1337.

TURTLE, E.E., TAYLOR, A., WRIGHT, E.N., THEARLE, R.J.P., EGAN, H., EVANS, W.H. and SOUTAR. N.M. : — The effects on birds of certain chlorinated insecticides used as seed dressings. *J. Sci. Fd Agri.* 14/8 (1963) 567.

SUMMARY

In a preliminary survey considerable amounts of chlorinated hydrocarbon insecticides were detected in several sea bird species in the Netherlands. To evaluate the probable toxicological implications of the residues found a special study was made of the sandwich tern during the summers of 1965 and 1966. From the results we conclude that in 1965 insecticides were responsible for the mortality observed among adult and junevile birds in the colony. It is considered that the decline in the Netherlands' population of this bird is due to poisoning from these insecticides. An account is given of the dispersion of these compounds in the coastal environment in the Netherlands from analysis of indicator organisms

SAMENVATTING

Insecticiden als factor in de mortaliteit van de grote stern (Sterna sandvicensis) in Nederland.

Bij een onderzoek naar het voorkomen van gechloreerde koolwaterstofbestrijdingsmiddelen in het natuurlijke milieu in Nederland werd gevonden, dat aanzienlijke hoeveelheden van deze stoffen voorkomen in bepaalde soorten zeevogels. Om de toxicologische betekenis van de gevonden residu's nader te toetsen is in de zomers van 1965 en 1966 een gericht onderzoek gewijd aan de grote stern. Uit de resultaten concluderen wij, dat enkele insecticiden in 1965 de dood hebben veroorzaakt van zowel adulte als juveniele vogels in de kolonie.

De achteruitgang van de Nederlandse grote stern-populatie wordt hiermee in verband gebracht. Er is een overzicht gegeven van de verspreiding van de gechloreerde koolwaterstof insecticiden in de kustwateren van Nederland aan de hand van analyses van indicator organismen.

RÉSUMÉ

La contribution des insecticides à la mortalité du Sterne caugek (Sterna sandvicensis) aux Pays-Bas.

Des recherches provisoires concernant la dispersion des insecticides dans le milieu naturel des Pays-Bas ont indiqué la présence de quantitées considérables de ces composées dans les tissus de diverses espèces d'oiseaux de mer. Une étude spéciale a été consacrée au Sterne caugek pendant les étés de 1965 et de 1966 afin de déterminer la signification toxicologique des résidus observés. Nous arrivons à la conclusion qu'en 1965 les insecticides organochlorés ont donné lieu à une mortalité aussi bien chez les adultes que chez les juvéniles. La relation entre le déclin de la population de ces sternes aux Pays-Bas et la mortalité par intoxication est discutée. La dispersion des insecticides dans le milieu marin à la cóte des Pays-Bas a été déterminée en utilisant les analyses d'organismes indicateurs.

ZUSAMMENFASSUNG

Insektizide als Faktor der Mortalität von Brandseeschwalben (*Sterna sandvicensis*) in den Niederlanden.

Während einiger vorläufigen Untersuchungen über die Verbreitung von chlorierten Kohlenwasserstoff-Bekämpfungsmitteln im natürlichen Milieu in den Niederlanden wurde gefunden, dass ziemlich grosse Mengen dieser Verbindungen vorkommen in den Geweben verschiedener Arten von Meeresvögeln. Um die toxikologische Bedeutung der gefundenen Rückständen festzustellen wurden spezielle Untersuchungen der Brandseeschwalbe in den Sommern 1965 und 1966 gewidmet. Wir schliessen daraus dass 1965 das Sterben von adulten und juvenilen Vögeln in der Seeschwalben-kolonie hauptsächlich verursacht wurde durch einige Insektizide. Der Rückgang der niederländischen Population der Brandseeschwalbe wird hiermit in Zusammenhang gebracht. Die Verbreitung dieser Insektizide an den Küsten der Niederlanden wird an Hand von Analysen von Indikator-Organismen erörtert.