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RESIDUES OF DDE AND PCB IN EGGS FROM HERRING GULL (LARUS ARGENTATUS) AND COMMON GULL (LARUS CANUS) IN NORWAY

By

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The use of organochlorine insecticides has been restricted almost exclusively to terrestrial areas. Nevertheless several recent investigations have revealed substantial concentrations of persistent insecticides in evertebrates and vertebrates in marine environments (*Moore & Tatton* 1965, *Koeman & van Genderen* 1966, *Risebrough et al.* 1967, *Holden & Marsden* 1967, *Robinson et al.* 1967, *Jensen et al.* 1969, *Prestt et al.* 1970). Residues of organochlorine insecticides have also been demonstrated in antarctic fauna (*Sladen et al.* 1966, *George & Frear* 1966) and in antarctic snow (*Peterle* 1969). This indicates a wide distribution of the persistent insecticides.

Upon examination of 256 birds (63 species) in Norway, residues of organochlorine insecticides were demonstrated in 49.6 % (*Holt & Sakshaug* 1968). The residues in most of the samples were below 1 p.p.m., except for the birds of prey. In these, the concentrations ranged from trace to 10–15 p.p.m. Most frequently encountered were residues of DDT, DDE and DDD while dieldrin and lindane residues occurred only sporadically. In a number of the tissue and egg samples analysed, varying concentrations of PCB-like compounds were demonstrated. Only a few of the birds examined were seabirds.

To elucidate the level of contamination by organochlorine insecticides and PCB in Norway's marine environments, eggs from

herring gull (Larus argentatus) and common gull (Larus canus) have been collected for analyses at different localities on the coastline from Oslo Fjord to Varanger Fjord.

MATERIAL AND METHODS

A total of 294 eggs from herring gull were collected at eight localities in Norway in 1969 (Fig. 1). The number of eggs collected at the different localities is given in Table 3. From each of the localities samples from 10 eggs were taken for analyses. At the Kragerø locality nine eggs from common gull were also collected and analysed.

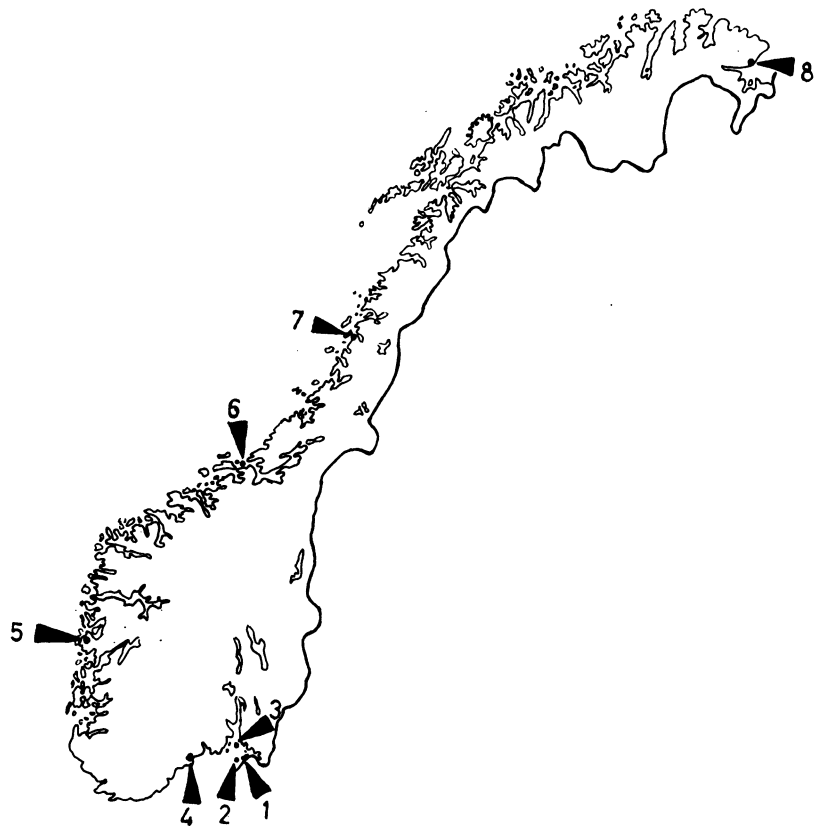

Figure 1. Map of Norway showing localities where the eggs were collected. The localities are: 1. Tisler, 2. Heia, 3. S. Missingen, 4. Kragerø, 5. Bergen, 6. Frøya/Tarva, 7. Sandnessjøen, and 8. Vadsø.

After removing the contents, the eggshells were allowed to dry at room temperature for several weeks. Then the thicknesses of eggshells (with outer membrane) were measured with a micrometer to the nearest hundredth of 1 mm. The eggshell thickness given in Table 3 is a mean of the thicknesses at four different points on the blunt end of the egg.

The contents of the eggs were homogenized and the samples frozen immediately and kept at –20° C until analyses could be performed. One g of the sample was then mixed with 1.5 g anhydrous magnesium sulphate and 1.5 g sand, and ground in a mortar.

The resultant dry, freeflowing powder was transferred to a small chromatographic column (12 mm Ø × 35–40 mm), previously filled with ethyl ether, and allowed to settle. To prevent the formation of air/vapor pockets, the column wall was tapped gently during the settling period. The column was eluted very slowly (approx. 0.2 ml/min.) with ethyl ether, and approx. 5 ml eluate were collected. After adjustment of the volume to 5.0 ml, two aliquots (A and B) of 2.3 ml each were taken from the eluate and evaporated to dryness.

To residue A were added 0.4 ml n-hexane and 1 ml concentrated sulphuric acid (94–97 %), and the mixture was shaken for 10 min. at ambient temperature.

Residue B was treated with 1.5 ml solution of 10 % potassium hydroxide in methanol at 40° C overnight, then shaken with 3 ml 2 % solution of sodium chloride in distilled water and 0.4 ml n-hexane for 10 min.

Both aliquots were run for 5 min. in a centrifuge at 1500 r.p.m. and subsequently frozen at approx. –70° C for 20 min. The hexane phases were decanted while still very cold and used for gas chromatographic determinations.

The method has been tested for recovery, using mussel as test sample. Standard solutions (100 µl) were added on the top of the extraction column, and the final volume of n-hexane used was 1 ml. Twenty parallels were run, and the results are given in Table 1.

Table 1. Results of 20 recovery tests.

Standards ng/ml		Per cent recovery	
		acid	base
BHC	10	107.4 s = 6.4	
Lindan	10	112.6 s = 9.2	
Aldrin	10	41.6	87.1 s = 21.3
Dieldrin	20		97.9 s = 8.1
DDE	20	103.2 s = 7.2	
DDD	50	100.3 s = 9.7	
DDT	80	117.7 s = 10.3	

s: Standard deviation.

The gas chromatographic equipment was as follows:

		Varian Aerograph
Equipment	Mod 205–1B	600 B/328 550 B/328
Columns	Borosilicate glass 2 mm (ID) × 180 cm silanized	Borosilicate glass 2 mm (ID) × 150 mm silanized
Column packings	QF-1 10 % on Chromosorb W AWHMDS 80/100 mesh	SF-96 4 % on Gaschrom P 80/100 mesh
Column temperature	175–180° C	170–175° C
Injector temperature	ca. 190° C	ca. 180° C
Detector temperature (in ionization tube)	ca. 150° C	ca. 130° C
Carrier gas	Nitrogen, highly purified, applied at pressures, adjusted to give DDT retention times of approx. 20 min.	

Both aliquots, A and B, were run on QF-1 and SF-96 columns, and the conclusions were drawn from the indications of the four chromatograms.

Preliminary tests showed large amounts of DDE, which made a 10–20-times dilution necessary. Only a few samples were run undiluted, even though the dilution resulted in a higher level of detection. Considering the additional work of running both diluted and undiluted samples, the small values of DDD and DDT were judged to be of little interest compared to the values of DDE.

The eggs contained appreciable amounts of polychlorinated biphenyls (PCB), which made the interpretation of chromatograms difficult. Dieldrin and/or DDD was not found. The detection of these compounds was made possible only by the differences in peak-heights on the four chromatograms, the detection limit being approx. 15 % of the DDE-value. DDT was detected in some samples on the QF-1 column, but this could not be confirmed on the SF-96 column, due to the interfering PCB.

All the PCB's from no. 4 (with retention volume on QF-1 as DDE), were present, with nos. 7, 8, and 10 as the major peaks (rel. ret. vol. to DDE on QF-1: 1.32, 1.41, and 1.77). It was supposed that the concentration ratios between these three PCB's were constant in this material.

The supposition was tested by analysing the SF-96 chromatograms of 11 random samples and of 11 parallels of one homogenate. In each chromatogram the peak-heights of PCB 7 and 8 were calculated relative to PCB 10, as shown:

Samples	Homogenate
PCB 7 70.2 % ± 10.4 %	63.5 % ± 12.1 %
PCB 8 144.5 % ± 14.8 %	139.5 % ± 8.6 %
PCB 10 100 %	100 %

The supposition seems to be correct within the reproducibility of the method. With minor corrections for differences in peak-height/concentration ratio, the concentration of PCB 7, 8, and 10 were assumed to be 3.16 times the amount of PCB 10, which was determined in all samples.

RESULTS

Substantial amounts of organochlorine insecticides and PCB have been found in this material. The results of the analyses, uncorrected for recovery, are presented in Table 2. In addition, four of these 80 eggs contained DDT, namely one from each of the following localities: Heia (0.15 p.p.m.), Bergen (0.18 p.p.m.), Sandnessjøen (0.16 p.p.m.), and Vadsø (0.30 p.p.m.). Other organochlorine insecticides were not detected.

In eggs from herring gull, DDE was found in concentrations between 0.2 and 5.4 p.p.m., while PCB 10 ranged between 0.2 and 3.8 p.p.m. There are significant differences in the amounts of

Table 2. Residues of DDE and PCB in eggs of herring gull (Larus argentatus) and common gull (Larus canus) collected in Norway 1969.

Species and locality	No. of eggs	Residue concentration in p.p.m. of wet weight						
		DDE			PCB 10			PCB 7, 8, 10 (calculated)
		mean	range	s	mean	range	s	
Herring gull								
1. Tisler	10	0.89	0.27—3.56	0.96	0.68	0.35—1.40	0.35	2.15
2. Heia	10	1.71	0.52—5.38	1.33	1.23	0.52—1.49	0.28	3.89
3. S. Missingen	10	1.18	0.43—2.34	0.74	1.03	0.32—2.11	0.54	3.25
4. Kragerø	10	2.12	0.80—3.75	1.09	1.56	0.61—3.76	0.98	4.94
5. Bergen	10	2.19	0.50—3.60	1.07	1.58	0.45—2.61	0.75	4.99
6. Frøya/Tarva	10	1.33	0.68—2.32	0.49	0.58	0.36—0.89	0.20	1.84
7. Sandnessjøen	10	1.12	0.64—1.79	0.35	0.63	0.29—1.83	0.44	1.99
8. Vadsø	10	1.69	0.87—2.41	0.47	0.74	0.25—1.62	0.44	2.35
Total	80	1.53	0.27—5.38	0.95	1.00	0.25—3.76	0.69	3.16
Common gull								
4. Kragerø	9	1.36	0.12—3.84	1.42	0.20	0.0 —0.84	0.28	0.63

s: Standard deviation.

residues between the localities (DDE: $P = 0.01$ and PCB: $P < 0.001$). There is a strong positive correlation ($r = 0.84$, d.f. $= 6$, $P = 0.01$) between the amounts of DDE and PCB when these are calculated on the basis of locality mean values.

It is seen from Table 2 that the eggs from herring gull evidently have higher residue levels of DDE and PCB than the eggs from common gull.

Table 3. Shell thickness (in 10^{-2} mm) of eggs from herring gull (Larus argentatus).

Locality	Collected eggs			Analysed eggs		
	no.	mean	s	no.	mean	s
1. Tisler	47	28.2	2.2	10	28.8	3.5
2. Heia	20	27.2	2.4	10	28.0	2.4
3. S. Missingen	17	28.3	2.9	10	27.5	2.7
4. Kragerø	40	27.0	1.9	10	27.6	1.7
5. Bergen	36	30.1	3.1	10	29.9	2.3
6. Frøya/Tarva	51	28.6	3.0	10	30.4	3.9
7. Sandnessjøen	44	28.9	2.3	10	31.3	2.8
8. Vadsø	39	30.7	2.3	10	30.4	2.4

s = Standard deviation.

The shell thicknesses of eggs from herring gull are shown in Table 3. Differences between the localities, concerning analysed eggs, have been found on the 5 % level only. The correlation of thickness to residue levels is low for DDE ($r = -0.12$, d.f. $= 6$, $P > 0.05$), but higher for PCB ($r = -0.54$, d.f. $= 6$, $P > 0.05$).

DISCUSSION

In this egg material the residue concentrations of organochlorine insecticides and PCB are considerably higher than what has been demonstrated previously in eggs and organ tissue of terrestrial birds in Norway (*Holt & Sakshaug* 1968). On the other hand the ratio DDT/DDE is extremely less than previously. This is in accordance with *Robinson et al.* (1967) who analysed species belonging to different tropic levels in marine food chains and demonstrated the presence of DDE simultaneously with DDT being either totally absent or present in concentrations below the limits of detection. From this evidence DDE seems to

be the chief component of organochlorine insecticides found in marine organisms.

PCB, which has some similarities in structure and properties to the DDT pesticide group, has very numerous and important industrial uses. These persistent organochlorines have been in common use for the last 40 years. Since *Jensen* (1966) reported their presence in organ tissue of wild animals, residues have been demonstrated also in different species of marine and terrestrial ecocystems (*Risebrough et al.* 1968, *Jensen et al.* 1969, and *Koeman et al.* 1969, *Prestt et al.* 1970).

The correlation between amounts of DDE and PCB in this egg material indicates that PCB may have the same pattern of distribution as the organochlorine insecticides.

Up to 1966, 9305 herring gulls, nearly all of them nestlings, had been banded in Norway. From the recoveries it appears that this bird species partly migrates within the local coastal areas and partly migrates to localities outside Norway. Most of the colonies in the southern parts of Norway winter in the country, but some birds may migrate as far south as Denmark. The colonies on the western coast of Norway are relatively stationary, while the colonies from the northern part of the country commonly migrate to continental Europe, often to the far south, further than southern colonies go when they migrate in the same direction. In Finnmark, the northernmost county of Norway, the herring gulls are typical migratory birds, and only a few birds winter in this locality. For the most part they migrate to continental areas of Denmark, to France and to localities on the coast of England and Scotland (*Haftorn* 1971).

It is reasonable to presume that only part of the DDE and PCB residue demonstrated in this material originated from local contamination. The rest must derive partly from global spread and partly from the body burden to which the birds have been exposed in other contaminated areas during their migration. Accordingly eggs from herring gull may not be suitable indicators of local conditions in northern Norway, but they may nonetheless be useful indicators in detecting general changes in environmental contamination by persistent organochlorines in the southern parts of the country.

Hickey et al. (1966) found that the concentration of total DDT-type material in the tissue of herring gull was independent of the age of the bird, but *Robinson et al.* demonstrated that in

eggs from shag (Phalacrocorax aristotelis) marked differences in the concentrations of organochlorine insecticides occurred within the breeding seasons. If such seasonal fluctuation also occurs in eggs from herring gull this may explain some of the differences found between localities concerning the amount of DDE. In this investigation it was not possible to coordinate the collection of eggs in the localities in relation to the same stages of breeding.

From Fig. 2 it appears that ranges in samples both concerning DDE and PCB are great and different in the different localities. This is in accordance with *Robinson et al.*, who found that in seabirds considerable variation in the concentrations of residues occurred in species of the same family and even within the same genus.

From Table 2 and Fig. 2 it is seen that in Heia and Vadsø, which represent the southernmost and northernmost localities respectively, the mean values of DDE are of the same order.

The total use of DDT in Norway has been comparatively low (10 tons in 1967), especially in the northern parts of the country. In addition environmental contamination by the universal spread of DDT and its metabolites is supposed to decrease at increasingly northern latitudes. The relatively high residue concentration in eggs from Vadsø and even from Sandnessjøen and Trondheim cannot be explained otherwise than that the herring gulls in these localities must have obtained some of their body burden of DDE from other contaminated areas during migration.

The highest values are found in eggs from Kragerø and Bergen. In previous examinations of Norwegian butter (*Sakshaug* 1968, *Bjerk & Sakshaug* 1969), high values of DDT and metabolites were found in samples from the Bergen area. Even though the fjords in the southwestern part of the country are fruit-growing districts, it is assumed that the global distribution is also responsible, especially for the PCB. The Atlantic winds deposit their rain in this mountainous area.

The feeding habits may explain the difference in residue concentrations of DDE and PCB found in eggs from herring gull and common gull (Table 2). By analysing stomach contents of northern European gulls, *Spärck* (1950) found that insects together with offal and vegetables were the principal food of common gulls, while fish, crustaceans, lamellibranchs, polychaetes, and other marine species were of less importance. In herring

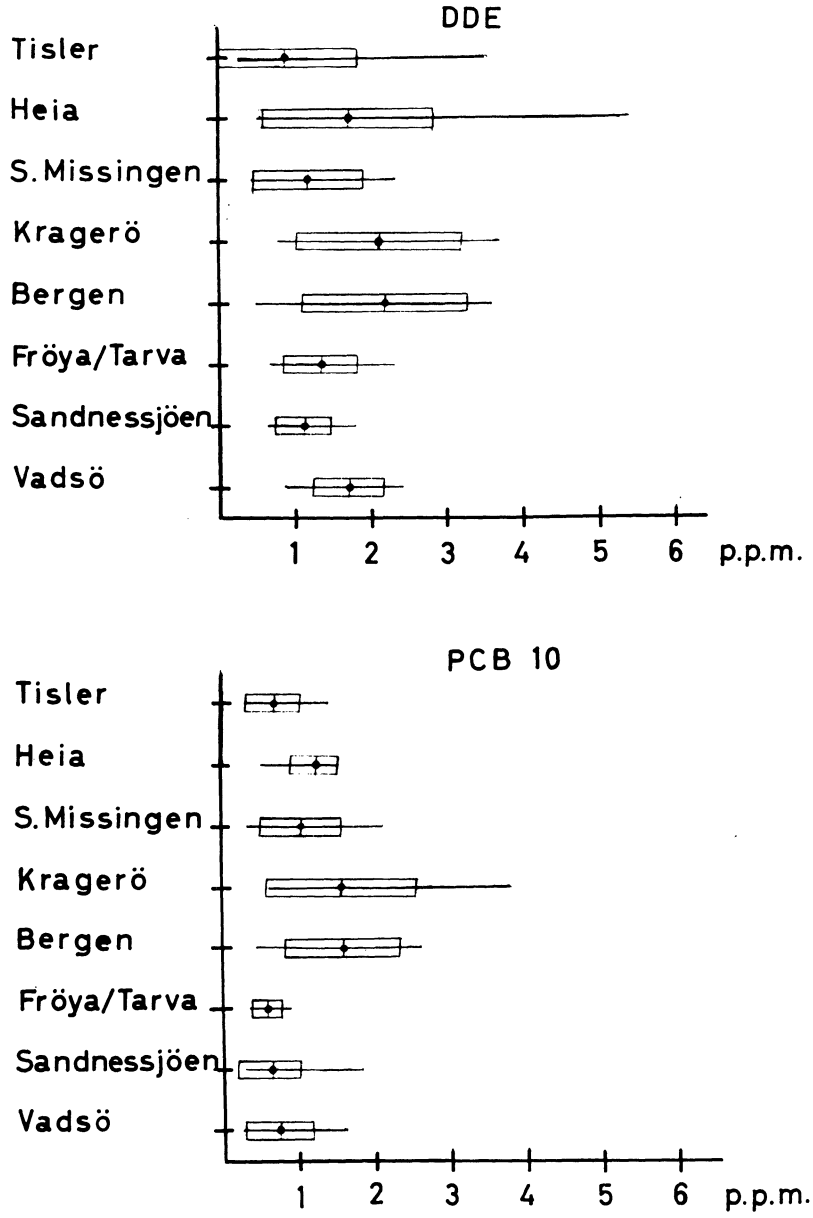

Figure 2. Residues of DDE and PCB 10 in eggs from herring gull (Larus argentatus) collected at different localities in Norway 1969. From each locality the mean value, standard deviation, and range are shown as a dot, a rectangle, and a line respectively.

gulls he found that insects were of very little importance while the chief components of food were fish and other marine organisms together with a very important amount of offal.

Since *Ratcliffe* (1967) threw light on the correlation between decrease in eggshell weight in certain birds of prey and exposure to persistent organochlorine insecticides, numerous investigations have been made concerning these relationships in different birds. *Hickey & Anderson* (1968) found that shell thickness in herring gull from five states in the USA decreased with increases in organochlorine insecticide residues. *Risebrough et al.* (1968) pointed out that PCB together with other chlorinated biocides are powerful inducers of hepatic enzymes which degrade oestradiol and that they can account for a large part of the abberation in calcium metabolism which has been observed in many species of birds during the last several years.

Eggshells vary greatly in thickness, a characteristic which is determined by numerous factors and is not always the same even in eggs layed by wild birds of the same species (*Romanoff & Romanoff* 1949). One of the factors that must be taken into account in relation to this investigation is the prevailing temperature of the different localities. *Warran & Schnepal* (1940) obtained thinner shells in eggs from Leghorn hens almost immediately after experimentally increasing the environmental temperature from 20° C to 32.5° C. A recovery in shell thickness occurred after a subsequent decrease in temperature.

The mean temperature in the breeding season of herring gull is 6° C higher in the Oslo Fjord than in the Varanger Fjord, which represent the southernmost and northernmost areas respectively from which the analysed eggs were collected.

It is suggested that differences in environmental temperatures and nutritional and other natural factors, more than effects from total content of DDE and PCB in the eggs, explain the differences between localities concerning the shell thickness.

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SUMMARY

In 1969 294 eggs from herring gull were collected from eight different localities in Norway. The eggshell thicknesses were measured, and 10 eggs from each locality were analysed by gas liquid chromatography for organochlorine insecticides and polychlorinated biphenyls (PCB).

Residues of DDE were demonstrated in all eggs, the concentrations varying from 0.2 to 5.4 p.p.m. in herring gull, and from 0.2 to 3.5 p.p.m. in common gull. DDT occurred only in four eggs from herring gull and then only in concentrations of from 0.1 to 0.3 p.p.m. Other organochlorine insecticides were not detected. Residues of PCB were found in all eggs from herring gull in concentrations of from 0.2 to 3.8 p.p.m. PCB 10, and in six out of nine eggs from common gull, from trace to 0.8 p.p.m. PCB 10.

The analysed material showed a positive correlation between amounts present of DDE and of PCB.

There was a significant difference between localities concerning the contents of DDE and PCB in eggs from herring gull. This variance may be taken into consideration by using the herring gull as an indicator organism, since this bird, especially in the northern part of Norway, is migratory.

The residue concentrations of DDE and PCB were markedly higher in eggs from herring gull than in eggs from common gull. It is suggested that this may be related to the difference between these species in their feeding habits.

The residues of organochlorines demonstrated in this investigation do not seem to have had any effect on eggshell thickness in herring gull.

SAMMENDRAG

Restkonsentrasjoner av DDE og PCB i egg fra gråmåke (Larus argentatus) og fiskemåke (Larus canus) i Norge.

I 1969 ble 294 egg fra gråmåke innsamlet på 8 forskjellige lokaliteter. Eggskalltykkelsen ble målt og homogenater av 10 egg fra hver lokalitet analysert gasskromatografisk med hensyn på rester av organiske klorinsekticider og polyklorerte bifenyler (PCB). Fra en av lokalitetene ble dessuten 9 egg fra fiskemåke analysert.

Rester av DDE ble påvist i alle egg i konsentrasjoner fra 0,2 til 5,4 p.p.m. hos gråmåke og fra 0,2 til 3,5 p.p.m. hos fiskemåke. DDT forekom bare i 4 egg fra gråmåke i konsentrasjoner fra 0,1 til 0,3 p.p.m. Andre organiske klorinsekticider ble ikke påvist. Rester av PCB ble

påvist i alle egg fra gråmåke i konsentrasjoner fra 0,2 til 3,8 p.p.m. PCB 10 og i 6 av 9 egg fra fiskemåke fra spor til 0,8 p.p.m. PCB 10.

I det samlede materiale var det en positiv korrelasjon mellom innholdet av DDE og PCB.

Det var en signifikant forskjell mellom de ulike lokaliteter med hensyn til restkonsentrasjonen av DDE og PCB i egg fra gråmåke. I Syd-Norge er gråmåken relativ stasjonær, mens den i de nordligste distrikter er en typisk trekkfugl, hvilket må tas i betraktning når det gjelder denne fuglearts anvendbarhet som indikatororganisme for de mer lokale forurensninger.

Restkonsentrasjonene av DDE og PCB var markert høyere i egg fra gråmåke enn fra fiskemåke. Det antas at dette skyldes de to arters forskjellige næringsvalg.

De påviste rester av organiske klorider synes ikke å ha hatt noen effekt på eggskalltykkelsen hos gråmåke.

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