

## Determination of Vitamin E in Aquatic Organisms by High-Performance Liquid Chromatography with Fluorescence Detection

Ji-Zeng Huo,\* Hans J. Nelis,† Patrick Lavens,‡ Patrick Sorgeloos,‡ and André P. De Leenheer\*,<sup>1</sup>

\*Laboratoria voor Medische Biochemie en voor Klinische Analyse and †Laboratorium voor Farmaceutische Microbiologie, Universiteit Gent, Harelbekestraat 72, B-9000 Gent, Belgium; and ‡Laboratory for Aquaculture, University of Gent, Rozier 44, B-9000 Gent, Belgium

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A liquid chromatographic (HPLC) method has been developed for the quantitative determination of different forms of vitamin E ( $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol) in aquatic organisms. The assay consists of a simple extraction with methanol containing butylhydroxytoluene (BHT) as an antioxidant, followed by reversed-phase chromatography with fluorescence detection. The efficiency of the extraction method was equivalent or superior to that of more complex approaches for the isolation of tocopherols. Linearity has been achieved over the range of 0.02 to 3  $\mu\text{g/ml}$  for  $\alpha$ -tocopherol and within-run and between-run coefficients of variation at three levels were 0.7–2.9 and 1.2–3.7%, respectively. The recovery at three concentrations ranged from 73.8 to 96.6% and the minimal quantity that could be detected was 0.6 ng. Comparable figures were obtained for  $\gamma$ - and  $\delta$ -tocopherol. This method has been routinely applied to determine vitamin E in *Artemia*, rotifers, turbot and sea bass larvae, and shrimp postlarvae. © 1996 Academic Press, Inc.

Vitamin E is essential to protect polyunsaturated fatty acids from peroxidation (1). In fish, deficiency in this nutrient leads to progressive damage of cellular and subcellular membranes, resulting in a variety of pathological signs (2). To improve the oxidative stability and, hence, the shelf life of fish, fortification of the feed with vitamin E is commonly applied in aquaculture. Accordingly, tocopherols are mainly quantitated in muscle of adult fish, e.g., salmon (3–7), catfish (8–10), carp (11), gilthead (12), albacore (13), sturgeon

(14), sea bass (15), and turbot (16). Few studies on vitamin E in aquatic organisms have been dealing with more fundamental aspects.

Current interest in our laboratories in the effects of this vitamin on the production characteristics, stress resistance, and susceptibility to disease of larval fish and shrimp prompted us to develop a new liquid chromatographic (HPLC) method for the determination of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol in these organisms as well as in their live food, i.e., *Artemia* and rotifers.

### MATERIALS AND METHODS

#### Chemicals and Reagents

$\alpha$ - and  $\gamma$ -tocopherol ( $\alpha$ -T,  $\gamma$ -T) were obtained from Eastman Kodak (Rochester, NY) and Acros Organic (Geel, Belgium), respectively.  $\delta$ -Tocopherol ( $\delta$ -T) and the internal standard tocol came from Eisai Co. (Tokyo, Japan). The chemical structures of the various tocopherols are depicted in Table 1. Stock solutions containing approximately 1–3 mg/ml as well as working solutions were prepared in methanol and stored at  $-20^{\circ}\text{C}$ . The exact concentrations were determined spectrophotometrically based on the  $E_{1\text{cm}}^{1\%}$  values from the literature. Methanol and hexane were HPLC grade and purchased from ROMIL (Loughborough, UK). Analytical grade butylhydroxytoluene (BHT)<sup>2</sup> and sodium dodecyl sulfate (SDS) were obtained from Merck (Darmstadt, Germany). Bond Elut CN cartridges (500 mg, 2.8 ml) came from Varian (Palo Alto, CA).

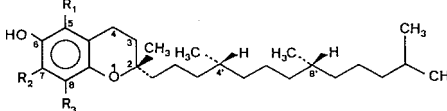
#### Samples

Nauplii of *Artemia franciscana* (Great Salt Lake strain) were produced from cysts using standard proce-

<sup>1</sup> To whom correspondence should be addressed. Fax: (32)9 264 81 97.

<sup>2</sup> Abbreviation used: BHT, butylhydroxytoluene.

TABLE 1  
Structures of Tocopherols

			
Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
α-Tocopherol	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>
γ-Tocopherol	H	CH <sub>3</sub>	CH <sub>3</sub>
δ-Tocopherol	H	H	CH <sub>3</sub>
Tocol	H	H	H

dures (17). Sea bass and turbot larvae, approximately 45 days old, came from the commercial hatcheries SEPIA (Gravelines, France) and France Turbot (Ile de Noirmoutier, France), respectively. A semipurified artificial fish powder (code 0271) was obtained from Rieber & Sons A/S (Bergen, Norway).

#### Apparatus and Chromatographic Conditions

The HPLC system consisted of a Varian 5020 pump (Varian, Palo Alto, CA), a Valco injector fitted with a 100-μl loop (Valco, Houston, TX), and a Perkin Elmer LS-4 fluorescence detector (Perkin Elmer, Norwalk, CT) with excitation at 296 nm and emission at 340 nm. A 15 × 0.46-cm 5 μ Hypersil ODS column (Shandon, Runcorn, UK) preceded by a 50 × 3-mm Chromguard reversed-phase guard column (Chrompack, Middelburg, The Netherlands) was eluted with a mixture of methanol:water (96:4, v/v) at a flow rate of 1 ml/min.

TABLE 2  
Recovery of Tocopherols from *Artemia* Using Different Procedures for Sample Preparation

Method <sup>a</sup>	Recovery <sup>b</sup> (±SD) % (n = 4)		
	α-T	γ	δ
1	100	100	100
2	100.5 ± 1.3	99.9 ± 1.3	95.2 ± 2.2
3	95.1 ± 1.5	94.5 ± 3.3	93.5 ± 4.5
4	48.0 ± 8.5	70.4 ± 5.4	88.9 ± 2.2
5	86.5 ± 1.5	88.5 ± 2.6	90.5 ± 2.8
6	98.7 ± 1.3	100.5 ± 1.0	102.0 ± 0.7

<sup>a</sup> 1, monophasic extraction with methanol; 2, saponification followed by double-phase extraction; 3, double-phase extraction with hexane before the addition of water; 4, double-phase extraction with hexane after the addition of water; 5, pretreatment with SDS and double-phase extraction; 6, homogenization in hexane-methanol.

<sup>b</sup> Relative to method 1 (=100%).

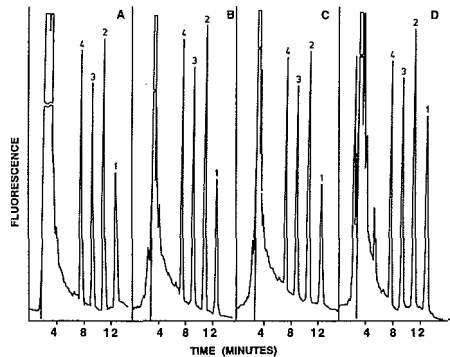


FIG. 1. Chromatograms obtained on extracts of turbot larvae supplemented with α-, γ-, and δ-tocopherol and tocol using different extraction methods. A, monophasic extraction with methanol; B, homogenization in hexane-methanol; C, double-phase extraction with hexane before the addition of water; D, saponification + double-phase extraction. Chromatographic conditions, see text. Peak identification: 1, α-tocopherol; 2, γ-tocopherol; 3, δ-tocopherol; 4, tocol (internal standard).

#### Sample Preparation: Standard Conditions

Samples of aquatic organisms (approximately 200 mg wet wt) were homogenized in 2 ml of methanol containing BHT (1 mg/ml) and the internal standard tocol, using a Potter Elvehjem tube or a Polytron mixer (Kinematica, Luzern, Switzerland). After centrifugation at 2000 rpm for 2 min, the supernatant was transferred to a polypropylene tube and the solid residue was reextracted with 2 ml of methanol-BHT. The extract was combined with the first one, as well as with a rinse (1 ml) of the Potter tube or the Polytron mixing tube. The final mixture was centrifuged at 10,000 rpm for 10 min and a 100-μl aliquot was injected.

#### Sample Preparation: Alternative Procedures

**Extraction with methanol followed by saponification and double-phase extraction.** After extraction of the sample under standard conditions, 1 ml of methanol containing 5% pyrogallol and 1.5 ml of 60% (w/v) aqueous potassium hydroxide were added and the mixture was saponified for 7 min at 70°C with periodic agitation of the tube. The cooled solution was extracted with 5 ml of a mixture of petroleum ether:diisopropyl ether (3:1, v/v) followed by the addition of 5 ml of water. After centrifugation, the upper layer was removed and evaporated to dryness under nitrogen at room temperature. The residue was redissolved in 5 ml of methanol-BHT and a 100-μl aliquot was injected.

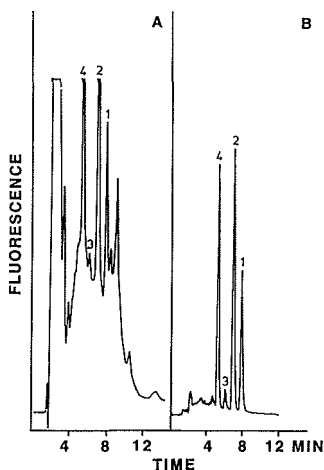


FIG. 2. Chromatograms of extracts of *Artemia nauplii* obtained by using two different extraction methods. A, monophasic extraction with methanol; B, homogenization in hexane-methanol. Conditions and peak identities were as described in the legend to Fig. 1.

*Extraction with methanol followed by reextraction with hexane.* The methanolic extract obtained with the standard procedure was reextracted with 5 ml of hexane either before the addition of 5 ml of water or after the addition of it. The hexane layer was evaporated to dryness and the residue was reconstituted as described above.

*Extraction with methanol-SDS followed by double-phase extraction.* This procedure was similar to the standard procedure but differed from it in that the first homogenization was carried out with a mixture of 1 ml of methanol-BHT (1 mg/ml) and 1 ml of aqueous 80 mM SDS. Methanol:water (1:1, v/v) containing BHT (1 mg/ml of methanol) (2 ml) was used for the second extraction and for rinsing the tube (2 ml). The mixture was extracted with 5 ml of hexane. The upper layer was evaporated to dryness under nitrogen at room temperature, the residue was reconstituted with 5 ml of methanol-BHT (1 mg/ml) and a 100- $\mu$ l aliquot was injected.

*Extraction with hexane-methanol.* Samples were homogenized using 3 ml of a mixture of hexane:methanol (2:1, v/v). After centrifugation and the transferring of the upper layer, the sample was rehomogenized with 2 ml of hexane followed by the addition of 1 ml of water to aid in the separation of the phases. To the combined supernatants another 1 ml of water was added to remove traces of methanol. The upper organic layer was

isolated and evaporated to dryness and the residue was reconstituted as described above.

#### Sample Purification

Crude extracts in methanol could be purified either by saponification or by double-phase extraction with hexane, as described above. Alternatively, a solid-phase cleanup was used. To this end, the primary methanolic homogenate was evaporated to dryness and the residue was reconstituted with 1 ml of hexane. The latter solution was applied on top of a Bond Elut CN cartridge (500 mg, 2.8 ml) which had been preconditioned with hexane. The cartridge was washed with 2 ml of hexane and elution was performed with 5 ml of hexane:isopropanol (97:3, v/v) containing 0.019% BHT. The eluate was evaporated to dryness and the residue was redissolved in 1 ml of methanol-BHT. Alternatively, the primary extract was prepared in hexane-methanol and the hexane layer was further processed as described above.

#### Quantitation

Quantitation was based on peak height ratios (analyte versus the internal standard tocol). Standardization was performed by analyzing samples (*Artemia* or sea bass larvae) supplemented with known quantities

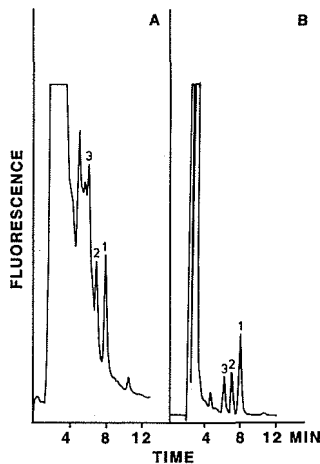


FIG. 3. Chromatograms obtained on fish feed using monophasic extraction and solid-phase purification. A, monophasic extraction with methanol; B, homogenization in hexane-methanol followed by purification on a cyanopropyl cartridge. Conditions and peak identities were as described in the legend to Fig. 1.

TABLE 3  
Recovery of Tocopherols from a Homogenate of Sea Bass Larvae

$\alpha$ -T Recovery			$\gamma$ -T Recovery			$\delta$ -T Recovery		
Concn ( $\mu\text{g/ml}$ )	%	SD%	Concn ( $\mu\text{g/ml}$ )	%	SD%	Concn ( $\mu\text{g/ml}$ )	%	SD%
0.095	73.8	8.6	0.052	87.8	2.8	0.054	83.4	2.7
0.75	94.7	1.6	0.42	98.4	1.0	0.42	96.1	1.0
3.02	96.6	2.3	1.67	99.8	2.2	1.74	97.8	2.3

of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -T and the internal standard tocol. Alternatively, in daily routine the analytes and the internal standard were added to methanol:water (96:4, v/v) and aliquots of these solutions were directly injected.

#### Method Validation

The recovery of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -T was determined by supplementing homogenized samples with each analyte and repetitively analyzing them using the standard procedure, except that the internal standard was added at the end. Extracts of the same sample to which both the analyte and the internal standard had been added just before the injection served as a reference (=100% recovery). To evaluate the linearity of the method, homogenates of fish or, alternatively, methanol were spiked (zero blind) with the analyte(s) and the internal standard, using 5 concentrations and 4 replicates per concentration for each tocopherol, according to the proposed NCCLS guideline (18). The within-day reproducibility was evaluated by analyzing 12 replicate samples at 3 levels, whereas for the day-to-day reproducibility 5 different levels of  $\alpha$ -T and one

level of  $\delta$ -T were used ( $n = 9$ ). The limits of quantitation for the different tocopherols were estimated from the analysis of spiked samples. The absolute detection limits (quantities injected) were determined using pure standard solutions. A peak height corresponding to three times the noise level was set as the limit of detectability.

#### RESULTS AND DISCUSSION

The selectivity of HPLC methods for the determination of tocopherols in biological materials (for reviews see Refs. 19–21) mainly depends on the detection mode (uv, fluorescence, or electrochemical detection) and on the extent of sample pretreatment. Because our method deals with the analysis of whole aquatic animals, the least selective technique, i.e., uv detection, was not considered. In accordance with many existing HPLC procedures for vitamin E in fish (6–14, 16, 22, 23) fluorescence detection was adopted. However, even with this more selective detection sample preparation is usually complex, including saponification (6–8, 10, 11, 13, 14, 16, 23) and/or double-phase extraction (9,

TABLE 4  
Reproducibility of the Determination of Tocopherols in Sea Bass and *Artemia*.

$\alpha$ -T			$\gamma$ -T			$\delta$ -T		
Concn, $\mu\text{g/ml}$	SD, $\mu\text{g/ml}$	CV, %	Concn, $\mu\text{g/ml}$	SD, $\mu\text{g/ml}$	CV, %	Concn, $\mu\text{g/ml}$	SD, $\mu\text{g/ml}$	CV, %
Within-run <sup>a</sup> ( $n = 12$ )								
0.015	0.00039	2.6	0.0084	0.00036	4.3	0.0083	0.00043	5.2
0.134	0.00301	2.2	0.074	0.00156	2.1	0.074	0.00149	2.0
0.530	0.00358	0.7	0.293	0.00095	0.3	0.292	0.00111	0.4
Between-run <sup>b</sup> ( $n = 9$ )								
0.024	0.00079	3.3				0.211	0.00158	0.8
0.061	0.00226	3.7						
0.152	0.00401	2.6						
0.304	0.00368	1.2						
0.608	0.01361	2.2						

<sup>a</sup> Obtained on spiked sea bass.

<sup>b</sup> Obtained on spiked *Artemia*.

12, 22). A simple monophasic extraction with a water-miscible solvent followed by the direct injection of an aliquot of the extract on the column has mainly been used for less complex, liquid samples such as plasma (24). We now demonstrate that this convenient, time-saving approach can also be applied to samples of aquatic organisms. Table 2 lists the comparative recoveries of three tocopherols from *Artemia* using different procedures, including monophasic extraction with methanol and methanolic extraction in conjunction with saponification and/or double-phase extraction. Two other extraction methods, i.e., pretreatment with sodium dodecyl sulfate prior to partitioning in hexane and direct homogenization in a mixture of hexane-methanol were also tried.

The performance of the monophasic approach was equivalent or superior to that of all other more complex procedures. Double-phase extraction from methanolic extracts yielded a satisfactory recovery of tocopherols (93.5–95.1%) only when water was added after their partitioning in hexane. In contrast, the addition of water before shaking with hexane resulted in an extraction yield of less than 50% for  $\alpha$ -T. Typical chromatograms obtained on extracts of *Artemia* using monophasic extraction, double-phase extraction, homogenization in hexane-methanol, and saponification + double-phase extraction are depicted in Fig. 1. The four chromatograms did not differ significantly in cleanliness and quantitation of all peaks posed no problems. However, certain samples of aquatic organisms containing low concentrations of vitamin E required higher sensitivity in order to detect the tocopherols in methanolic extracts. These extracts sometimes showed a huge front peak that could interfere with the peak of tocopherols. In those cases a purification by double-phase extraction or direct homogenization in hexane-methanol (Fig. 2) would be of interest. Further purification can be performed by solid-phase cleanup on a cyanopropyl cartridge, as illustrated in Fig. 3 for an extract of fish feed. The total recovery of  $\alpha$ -tocopherol from fish feed using a primary extraction with methanol, back-extraction in hexane, and solid-phase cleanup averaged  $70.3 \pm 3.9\%$  (concentration range 0.03–0.3  $\mu\text{g}/\text{ml}$  of final extract,  $n = 4$ ). This more complex sample preparation proved useful for the analysis of certain fish feeds containing low concentrations of vitamin E, i.e., on the order of 1.5–14.6  $\mu\text{g}/\text{g}$ .

The addition of antioxidants during sample preparation in assays of vitamin E is a matter of controversy (19). It is generally recognized that this protective measure is required for samples that contain substances promoting the oxidation of tocopherols, e.g., red blood cells (19). We found comparable recoveries of  $\alpha$ -T from fresh fish samples, irrespective of the presence of the antioxidant BHT. However, when a particular freeze-dried fish powder was analyzed, the recovery of  $\alpha$ -T

was below 10% in the absence versus  $89.6 \pm 1.2\%$  in the presence of BHT. Surprisingly, the corresponding figures for  $\gamma$ -T and  $\delta$ -T differed less (78.8 versus 95.2 and 98.0 versus 99.5, respectively).

The present HPLC method in conjunction with a monophasic extraction has been validated with respect to linearity, recovery, and reproducibility. A comparison with an established method for the determination of vitamin E in aquatic organisms was not made because such reference procedures were not available. Linearity was achieved over the concentration range of 0.023–3.0  $\mu\text{g}/\text{ml}$  ( $\alpha$ -T) and 0.013–1.67  $\mu\text{g}/\text{ml}$  ( $\gamma$ -T and  $\delta$ -T), respectively, with correlation coefficients exceeding 0.999. A comparison of nine calibration curves obtained by extracting spiked *Artemia* samples and by injecting standards, including tocopherol, in methanol:water (96:4, v/v), respectively, revealed no significant difference in slope ( $0.02076 \pm 0.00074$  versus  $0.02059 \pm 0.00021$ ) ( $P = 0.516$ , paired  $t$  test). Hence, the equivalency of both standardization approaches warranted the routine use of the simpler direct injection procedure. Data on recovery and reproducibility are listed in Tables 3 and 4. The detection limits (absolute quantities of pure analytes injected) were 0.8, 0.4, and 0.3 ng for  $\alpha$ -T,  $\gamma$ -T, and  $\delta$ -T, respectively. The assay is now routinely applied to monitor attempts to enrich fish larvae in vitamin E, based on evidence suggesting that higher vitamin E levels possibly lead to improved production characteristics and stress resistance of larval fish. These results will be reported elsewhere.

#### ACKNOWLEDGMENT

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#### REFERENCES

1. Bourgeois, C. (1992) Determination of Vitamin E: Tocopherols and Tocotrienols, p. 6, Elsevier, New York.
2. Halver, J. E. (1989) in *Fish Nutrition* (Halver, Jr E., Ed.), pp. 91–93, Academic Press, San Diego.
3. Cowey, C. B., Adron, J. W., Walton, M. J., Murray, J., Youngson, A., and Knox, D. (1981) *J. Nutr.* **111**, 1556–1567.
4. Cowey, C. B., Adron, J. W., and Youngson, A. (1983) *Aquaculture* **30**, 85–93.
5. Pozo, R., Lavety, J., and Love, R. M. (1988) *Aquaculture* **73**, 165–175.
6. Frigg, M., Prabucki, A. L., and Ruhdel, E. U. (1990) *Aquaculture* **84**, 145–158.
7. Sigurgisladottir, S., Parrish, C. C., Ackman, R. G., and Lall, S. P. (1994) *J. Food Sci.* **59**, 256–259.
8. Erickson, M. C. (1991) *J. Food Sci.* **56**, 1113–1114.
9. Bai, S. C., and Gatlin, D. M. (1993) *Aquaculture* **113**, 129–135.
10. Erickson, M. C. (1993) *J. Agric. Food Chem.* **41**, 1213–1218.
11. Runge, G., Steinhart, H., Schwarz, F. J., and Kirchgessner, M. (1992) *J. Anim. Physiol. Anim. Nutr.* **67**, 16–24.

12. Cabrini, L., Landi, L., Stefanelli, C., Barzanti, V., and Sechi, A. M. (1992) *Comp. Biochem. Physiol. B* 101, 383-386.
13. Pozo, R. G., Saitua, E. S., Uncilla, I., and Montoya, J. A. (1990) *J. Food Sci.* 55, 77-78.
14. Agradi, E., Abrami, G., Serrini, G., McKenzie, D., Bolis, C., and Bronzi, P. (1993) *Comp. Biochem. Physiol. A* 105, 187-195.
15. Stephan, G., Messenger, J. L., Lamour, F., and Baudin Laurencin, F. (1993) in *Fish Nutrition in Practice* (Kaushik, S. J., and Luquet, P., Eds.), pp. 215-218, INRA Editions, Versailles, France.
16. Stephan, G., Guillaume, J., and Lamour, F. (1995) *Aquaculture* 130, 251-268.
17. Lavens, P., and Sorgeloos, P. (1996) *Manual on the Production of Live Food for Aquaculture*, Food and Agriculture Organization, Rome, Italy.
18. Passey, R. B., Bee, D. E., Caffo, A., and Erikson, J. M. (1986) *Evaluation of the Linearity of Quantitative Analytical Methods*, NCCLS Document EP6-P, 6(18).
19. De Leenheer, A. P., Nelis, H. J., Lambert, W. E., and Bauwens, R. M. (1988) *J. Chromatogr.* 429, 3-58.
20. Nelis, H. J., De Bevere, V. O. R. C., and De Leenheer, A. P. (1985) in *Modern Chromatographic Analysis of the Vitamins* (De Leenheer, A. P., Lambert, W. E., and De Ruyter, M. G. M., Eds.), pp. 129-200, Dekker, New York.
21. Lang, J. K., Schillaci, M., and Irvin, B. (1992) in *Modern Chromatographic Analysis of Vitamins* (De Leenheer, A. P., Lambert, W. E., and Nelis, H. J., Eds.), 2nd ed., pp. 153-195, Dekker, New York.
22. Hung, S. S. O., Cho, Y. C., and Slinger, S. J. (1990) *J. Assoc. Off. Anal. Chem.* 63, 889-893.
23. Indyk, H. E. (1988) *Analyst* 113, 1217-1221.
24. Zaspel, B. J., and Csallany, A. S. (1983) *Anal. Biochem.* 130, 146-151.

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## Atmospheric Concentrations and Deposition of Heavy Metals over the North Sea: A Literature Review

JASNA INJUK and RENÉ VAN GRIEKEN

*University of Antwerp (U.I.A.), Department of Chemistry, B-2610 Antwerpen-Wilrijk, Belgium*

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**Abstract.** A literature review of the atmospheric concentration rates and dry and wet deposition fluxes of particulate Cd, Cr, Cu, Pb and Zn to the North Sea and adjacent areas is given. The results of direct measurements of dry and wet deposition fluxes are compared to indirect estimates and to modelling values. This work points out the large uncertainties in results of different studies on atmospheric input of trace elements into the North Sea. The current knowledge about the dependence of the deposition velocity upon the particle size and about the processes controlling wet deposition fluxes, and the quality and completeness of the emission data are still inadequate for describing the environmental cycle and impact of heavy metals in the North Sea.

**Key words:** Heavy metals, concentration, deposition, North Sea, troposphere.

### 1. Introduction

At present, there is much discussion on the sources and abatement policies of pollution to the sea. Atmospheric transport and deposition of pollutants over long distances have received much attention, particularly in connection with the acid rain problem, the formation of photochemical oxidants and ozone, and the global climatic effects. It is only relatively recently that it has become possible to estimate the amounts of material entering the oceans via the atmosphere. Though the pollution of the oceans is not a new phenomenon, the question of how important a role the atmosphere plays in this was addressed only a decade ago (NAS, 1978). As the calculations have become less crude, the atmospheric route seems to have gained in importance relative to the other paths, like those borne by rivers and direct discharges.

Still, quantitative data on heavy metal emissions, their concentration levels in air and their accumulation and transfer in the North Sea ecosystem have not been systematically gathered and intercompared hitherto. There are various sources which emit trace metals into the atmosphere, i.e. man-made pollution, aerosol formation from sea-spray, volcanic activity, vegetation and soil erosion. The concentrations of anthropogenic pollutants in the atmosphere are mainly due to the volatility of these elements at the high temperatures of fossil fuel combustion, and many other high-temperature industrial processes, particularly the extraction of non-ferrous metals