Anal. Chem. **2002,** *74,* 6121−6125

Measurements of Chemical Warfare Agent Degradation Products Using an Electrophoresis Microchip with Contactless Conductivity Detector

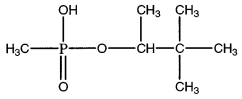
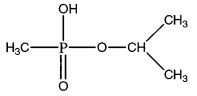
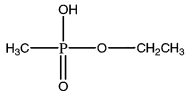
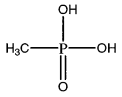
Joseph Wang,*,† Martin Pumera,† Greg E. Collins,‡ and Ashok Mulchandani§

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003, Chemistry Division, Naval Research Laboratory, Washington D.C. 20375, and Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521

This paper reports on a microfluidic device for the screening of organophosphonate nerve agent degradation products. The miniaturized system relies on an efficient chip-based separation of alkyl methylphosphonic acids (breakdown products of Sarin, Soman, and VX nerve agents) followed by their sensitive contactless conductivity detection. Experimental parameters relevant to the separation and detection processes have been optimized to yield high sensitivity (with 48−86 μg L^{-1} detection limits), fast response (50 s for a three alkyl methylphosphonic acid mixture), high precision (RSD = 3.8−5.0%), and good linearity (over the 0.3−100 mg L^{-1} range). Applicability to natural (river) water samples is demonstrated. The new microsystem offers promise for monitoring degradation products of chemical warfare agents, with advantages of speed/warning, efficiency, portability, sample size, and cost compared to conventional ion chromatography or capillary electrophoresis systems.

Nerve agents derive their name from their adverse effect on the nervous system. In response to recent terrorist activity and to the ratification of the Chemical Warfare Convention, there are urgent demands for rapid and reliable methods for the determination of chemical warfare agents (CWA) and their degradation products. For example, there are growing needs for developing field-screening methods capable of detecting organophosphonate nerve agents breakdown products.[1,2] Three such common agents, Sarin, Soman, and VX, contain the alkyl methylphosphonate moiety. Rapid identification of their degradation products will allow first responders to make the important decisions concerning evacuating or decontaminating of an attack site and will prevent them from becoming victims themselves. Additionally, such detection capability is crucial for verifying the production and destruction of organophosphonate nerve agents. Since alkyl methylphosphonic acids (Table 1)) are much more stable in the environment than their corresponding parent nerve agents, these compounds are commonly used for indicating the presence or use of organophosphonate CWA agents.[2]

Table 1. Hydrolsis Products of Nerve Agents

CWA degradation product	Structure of CWA degradation product	Corresponding CWA
PMPA (pinacolyl methylphosphonic acid)		Soman (GD)
IMPA (isopropyl methylphosphonic acid)		Sarin (GB)
EMPA (ethyl methylphosphonic acid)		VX
MPA (methylphosphonic acid)		Soman, Sarin, VX

Measurements of alkyl methylphosphonic acid nerve agent degradation products is challenging because they have no chromophore or fluorophore for UV or fluorescence detection. Ion chromatography has been traditionally used for monitoring these CWA products.[1,2] More recently, conventional capillary electrophoresis (CE) systems based on indirect UV[3,4] or conductivity[3−5] detection modes have been introduced for monitoring breakdown products of organophosphonate nerve agents.

This note describes a chip-based CE/conductivity microfluidic device for fast screening of CWA degradation products. "Lab-on-a-chip" technology offers tremendous potential for obtaining the

* Corresponding author. E-mail: joewang@nmsu.edu .
† New Mexico State University.
‡ Naval Research Laboratory.
§ University of California, Riverside.

(1) Bossle, P. C.; Reutter, D. J.; Sarver, E. W. *J. Chromatogr.* **1987**, *407*, 397.
(2) Kingery, A. F.; Allen, H. E. *Anal. Chem.* **1994**, *66*, 155.
(3) Nassar, A.-E. F.; Lucas, S. V.; Jones W. R.; Hoffland, L. D. *Anal. Chem.* **1998**, *70*, 1085.
(4) Nassar, A.-E. F.; Lucas, S. V.; Myler, C. A.; Jones, W. R.; Campisano, M.; Hoffland, L. D. *Anal. Chem.* **1998**, *70*, 3598.
(5) Rosso, T. E.; Bossle, P. C. *J. Chromatogr., A* **1998**, *824*, 125.

10.1021/ac025746p CCC: $22.00
Published on Web 11/01/2002

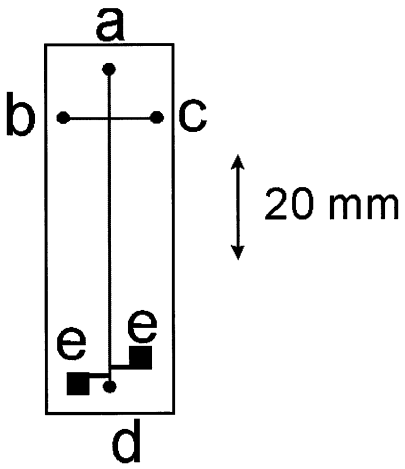


Figure 1. Top view of the microchip electrophoretic system with the contactless conductivity detection: run buffer reservoir (a), unused reservoir (b), sample reservoir (c), outlet reservoir (d), and aluminum sensing electrodes (e).

desired forensic information in a faster, simpler, and less costly manner compared to traditional laboratory-based instruments. Particularly attractive for on-site counterterrorism and compliance applications is the high-performance, small-footprint, high-throughput, versatility of microchip devices. CE microchips, based on amperometric[6] and fluorescence[7] detection, have been recently employed for monitoring organophosphorus pesticides. Yet, analogous chip-based assays of organophosphonate CWA breakdown products have not been reported. The present CWA microchip system combines the distinct advantages of contactless conductivity detection[8,9] with the attractive features and disposability of plastic CE microchips.[10,11] Such contactless conductivity detection offers various advantages for separation chips, including the elimination of surface fouling or bubble formation, effective isolation from high separation voltages, simplified detector design and electrode alignment, and use of narrow microchannels. The resulting chip-based monitoring of major nerve agent breakdown products is advantageous in terms of speed, miniaturization/portability, efficiency, cost, sample size, or simplicity compared to conventional ion chromatography and CE systems and, hence, holds promise for addressing the needs of major defense scenarios.

EXPERIMENTAL SECTION

Apparatus. The plastic microchip, shown in Figure 1, consisted of two sealed PMMA plates (70 × 24 mm) with a 51-mm-long separation channel (between the injection port and the outlet reservoir) and 18-mm-long injection channel (between the sample reservoir and the unused reservoir). The bottom plate (containing microfluidic network) and the cover plate had a thickness of 1 mm and 125 μm, respectively. The two channels crossed each other halfway between the sample and the unused reservoirs, at 9 mm from the run buffer reservoir. The channels had a 50 μm × 50 μm squared cross section. The PMMA microchips were fabricated at the Institut für Mikrotechnik Mainz (IMM, Mainz, Germany) and were described earlier.[10]

The rectangular-shaped electrodes (0.8 mm × 10 mm) were fabricated from two 10-μm-thick aluminum foil strips. The end side of the electrode was widened to 4 mm to facilitate the electrical connection. The electrodes were fixed to the top of the 125-μm-thick PMMA cover plate of the microchip using a common epoxy, at a distance of 600 μm from the end of microchannel. The thin copper wires (0.1 mm diameter, 15 mm long) were attached to the electrodes using a conducting epoxy (Chemtronics, Kennesaw, GA) and were tin-soldered to the detector electronics. The electrodes were separated by 700-μm distance and were placed in an "antiparallel" orientation to minimize the stray capacitance between them (Figure 1e). The electronic circuitry of the contactless conductivity detector, placed on the top of the chip, was identical to a previously reported one.[9] This scheme allows convenient interface to data acquisition systems (chart recorder or a computer DAQ using LabView 6i). The electronic components were purchased from local suppliers. The electronic circuit was placed in a shielded box to protect it from external electric fields. The open side of the box was placed (on the chip) as close as possible to the sensing electrodes, so that the box acted also as a shield for the electrodes. Further minimization of the noise was achieved by securing the chip (along with the printed electronic board) from possible mechanical vibrations. A HP 8116A function generator (Hewlett-Packard, Palo Alto, CA) was used for generating the ac signal (usually a sinus waveform with a frequency of 300 kHz and peak-to-peak amplitude of 5 V_{p-p}).

The homemade high-voltage power supply had an adjustable voltage range between −4000 and +4000 V. A homemade Plexiglas holder was used for supporting the separation chip. Short pipet tips were inserted into each of the four reservoir holes on the PMMA chip to provide solution contact between the channel on the chip and corresponding reservoir on the chip holder.

Reagents. Histidine (His) and 2-(*N*-morpholino)ethanesulfonic acid (MES) were purchased from Sigma. Methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), pinacolyl methylphosphonic acid (PMPA), methanol, sodium borate, boric acid, tris(hydroxymethyl)aminomethane (Tris), and hydrochloric acid were obtained from Aldrich, while isopropyl methylphosphonic (IMPA) acid was purchased from Cerilliant (Austin, TX). Stock solutions (10 000 mg L^{-1}) of the alkylphosphonic acids (MPA, EMPA, PMPA, IMPA) were prepared in methanol. All chemicals were used without further purification.

The electrophoretic buffer was a MES/His buffer (usually 5 mM, pH 6.1), prepared by dissolving MES and His in deionized water. Borate and Tris buffers were used for comparison (both at concentration of 20 mM, pH 6.1; the pH was adjusted using 20 mM boric acid and concentrated hydrochloric acid, respectively). The river water sample was collected from the Rio Grande River in Las Cruces, NM. The pH of the sample was adjusted to 6.1 by mixing 9.0 mL of river water and 1.0 mL of the MES/His buffer (50 mM) to give a final MES/His concentration of 5 mM. The

(6) Wang, J.; Chatrathi, M. P.; Mulchandani, A.; Chen, W. *Anal. Chem.* **2001**, *73*, 1804.
(7) Hadd, A. G.; Jacobsson, S. C.; Ramsey, J. M.; *Anal. Chem.* **1999**, *71*, 5206.
(8) Zemann, A. J. *Trends Anal. Chem.* **2001**, *20*, 346.
(9) Pumera, M.; Wang, J.; Opekar, F.; Jelínek, I.; Feldman, J.; Löwe, H.; Hardt, S. *Anal. Chem.* **2002**, *74*, 1968.
(10) Wang, J.; Pumera, M.; Chatrathi, M. P.; Escarpa, A.; Konrad, R.; Griebel, A.; Dörner, W.; Löwe, H. *Electrophoresis* **2002**, *23*, 596.
(11) Boone, T. D.; Fan, Z. H.; Hooper, H. H.; Ricco, A. J.; Tan, H.; Williams, S. J. *Anal. Chem.* **2002**, *74*, 78A.

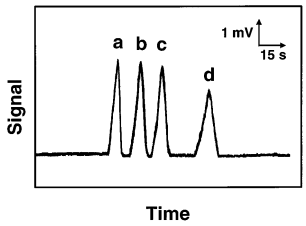


Figure 2. Electropherogram for a mixture of CWA degradation products: (a) 15 ppm MPA, (b) 20 ppm EMPA, (c) 20 ppm IMPA, and (d) 50 ppm PMPA. Conditions: separation buffer, 5 mM MES/His (pH 6.1); separation field strength, −250 V/cm; injection field strength, −250 V/cm; injection time, 2 s; frequency, 300 kHz; peak-to-peak voltage, 5 V_{p-p}; sinus ac waveform.

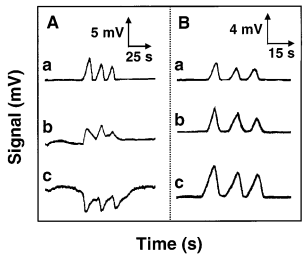


Figure 3. Effect of the run buffer (A) and its concentration (B) upon the separation and response of MPA, EMPA, and IMPA (in the order of appearance: concentrations, 40 ppm for A, 20 ppm for B). (A) Buffers tested: MES/His (a), Tris (b), and borate (c) buffers (20 mM, pH 6.1). (B) Run buffer (MES/His, pH 6.1) concentrations: (a) 20, (b) 10, and (c) 5 mM. Other conditions, as in Figure 2.

river water sample was filtered with an Acrodisc syringe filter (0.45 μm; Pall Corp.).

Electrophoresis Procedure. The channels of the plastic chip were treated before use by rinsing with deionized water for 10 min. Reservoirs a, b, and d (Figure 1) were filled with the electrophoretic run buffer solution, while reservoir c was filled with the sample mixture (analytes dissolved in the run buffer). After the initial sample loading (in the injection channel), the sample was injected by applying a field strength of −250 V/cm for 2 s between the sample reservoir c and the grounded outlet reservoir d. This drove the sample "plug" into the separation channel through the intersection. The analytical separation was performed by applying the separation potential to the run buffer reservoir a with the outlet reservoir d grounded and all other reservoirs floating.

Safety Considerations. *The high-voltage power supply should be handled with extreme care to avoid electrical shock. Alkyl methylphosphonic acids are extremely toxic and destructive to tissues. Other used chemicals are irritants to the skin; eye contact and accidental inhalation or ingestion should be avoided.*

RESULTS AND DISCUSSION

The present study targeted priority CWA degradation products, such as PMPA, IMPA, EMPA, and MPA (corresponding to Soman, Sarin, and VX nerve agent, respectively; Table 1), in connection with a recently developed plastic CE microchip coupled with contactless conductivity detector.[9] Figure 2 displays a typical electropherogram recorded with the new microsystem for a mixture containing parts-per-million (ppm) concentrations of MPA (a), EMPA (b), IMPA (c), and PMPA (d). Well-defined and resolved peaks are observed for all four anions. The peak half-width ranges from 4.1 (MPA) to 6.8 s (PMPA). Such a defined response, coupled to the flat baseline and low noise level, offers convenient quantification of ppm levels of all four alkyl methylphosphonic acids within 150 s. (Faster assays of lower concentrations are described below.) Two such products (and peaks)—a specific alkyl methylphosphonic acid and a universal methylphosphonic acid—are always expected based on the degradation pathway of CWA (Table 1), hence providing the necessary confirmation and signature. The separation of CWA degradation products was carried out in the anodic mode, using a MES/His run buffer (5 mM, pH 6.1), without adding an electroosmotic flow reversor/suppressor. Such capability is attributed to the favorably low electroosmotic flow of the PMMA microfluidic device ($\mu_{EOF} = 1.06 \times 10^{-4}$ cm^2/V·s, in our case). The absence of an EOF modifier leads to a lower background conductivity and therefore to lower detection limits.

Various operational parameters influencing the response were examined and optimized. The composition of running buffer has a profound effect upon the CE microchip separation of CWA degradation products (Figure 3). A MES/His buffer (Figure 3A, a) offers higher resolution and a more sensitive response for the alkyl methylphosphonic acids compared to Tris and borate buffer solutions (Figure 3A, b and c, respectively). The negative peaks in borate buffer correspond to the higher mobility of the running buffer co-ions compared to the analyte mobilities.[8,12] (Note that the buffers used here have different ionic strengths, and hence, the observed effects should be taken only qualitatively.) Figure 3B displays the influence of the running buffer concentration upon the conductivity signal. Lowering the running buffer concentration from 20 to 5 mM (and hence of the background conductivity) resulted in a 2-fold increase of the MPA peak (a vs c). A similar behavior was observed in conventional CE conductivity systems.[13,14] The migration times (and hence the resolution) were not affected by the change in buffer concentration.

The effect of the separation field strength upon the detector output was studied. As expected, increasing the separation field from −83 to −667 V/cm dramatically decreases the migration time for MPA, EMPA, and IMPA from 251 to 31, from 285 to 37, and from 312 to 42 s, respectively (not shown). The shorter migration times observed at higher fields are coupled to substantially sharper peaks. A separation field −667 V/cm thus allowed the separation of the three CWA degradation products within 50 s. Substantially longer (3−5 min) periods are required for complete separations using conventional capillary electrophoresis or ion chromatography systems.[2,3] The plate number for the three CWA degradation products thus increased from 1353 to 1740 (MPA), from 1390 to 2478 (EMPA) and from 1668 to 3194 (IMPA) over −83 to −667 V/cm range. The separation field strength had a negligible effect upon the peak-to-peak background noise level for field strengths ranging from −83 to −500 V/cm. Higher separation fields resulted in higher background and noise levels (attributed to Joule heating effects).

As expected, the response of the contactless conductivity detector is strongly dependent upon the waveform and frequency

(12) Ackermans, M. T.; Everaerts, F. M.; Beckers, J. L. *J. Chromatogr.* **1991**, *549*, 345.
(13) Huang, X.; Gordon, M. J.; Zare, R. N. *J. Chromatogr.* **1989**, *480*, 285.
(14) Huang, X.; Zare, R. N. *Anal. Chem.* **1991**, *63*, 2193.

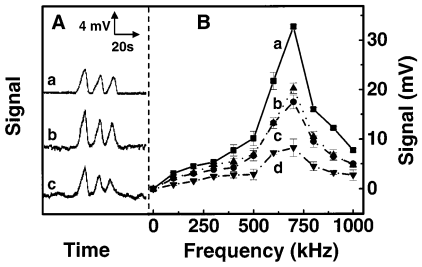


Figure 4. Influence of ac voltage upon the detector response. (A) Effect of waveform upon the response for 20 ppm MPA, EMPA, and IMPA. Sine (a), square (b), and triangle (c) waveforms, frequency 300 kHz, peak-to-peak voltage $5V_{p-p}$. (B) Effect of the applied ac voltage frequency upon the response. Sample contained 50 ppm MPA (a), EMPA (b), IMPA (c), and PMPA (d). Other conditions, as in Figure 2.

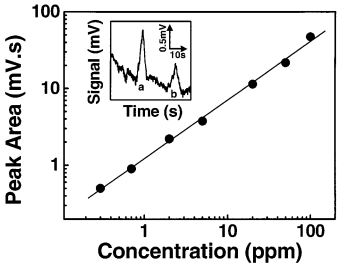


Figure 5. Dependence of the peak area on the IMPA concentration. Conditions, as in Figure 2. Also shown, as inset, is the response for a mixture containing 150 ppb MPA (a) and IMPA (b) using an ac frequency 700 kHz; other conditions, as in Figure 2.

of the applied voltage (Figure 4). Figure 4A compares electrophoregrams obtained using sine (a), square (b), and triangle (c) waveforms at a frequency of 300 kHz and peak-to-peak voltage of 5 V_{p-p}. The sine wave offers the most favorable signal-to-noise characteristics. The square and triangle waveforms resulted in higher signals than the sine waveform (e.g., 1.6- and 1.2-fold, respectively, for MPA) but in connection with a substantially larger noise level. Figure 4B shows the influence of the applied ac frequency upon the response of CWA degradation products. All four nerve agent breakdown products exhibit a similar trend. The response increases slowly between 100 and 500 kHz, then more rapidly, reaching the maximum at 700 kHz, and decreases sharply thereafter. Yet, an unstable baseline and response were observed over the 400−800-kHz range (as indicated from the larger error bars). It is clear that low frequencies lead to a more well-defined and stable response. However, the most favorable signal-to-noise ratio was obtained at frequency 700 kHz. Most subsequent work employed a frequency of 300 kHz that offered the most favorable response characteristics.

The contactless conductivity microchip detector displays a well-defined concentration dependence for CWA degradation products. A linear range of nearly 3 orders of magnitude (from 300 ppb to 100 ppm; correlation coefficient, 0.998) was obtained for IMPA (Figure 5). Detection limits of 48 (MPA) and 86 ppb (IMPA) can be estimated from the signal-to-noise characteristics (S/N = 3) of the response for a mixture containing 150 ppb of these CWA degradation products (Figure 5, inset). Such detection limits compare favorably with those (75−100 ppb) reported for conventional CE systems with indirect UV or conductivity detection.[3,5] Further lowering of detection limits is expected with connection

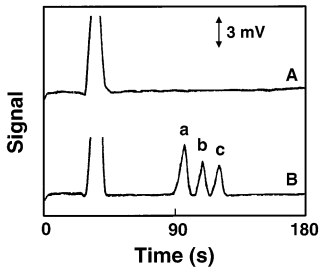


Figure 6. Electrophoregram for river water (Rio Grande) sample before (A) and after (B) the addition of 20 ppm MPA (a), EMPA (b), and IMPA (c). The untreated sample was filtered and spiked with the required amounts of MES/His puffer (to yield 5 mM level and pH 6.1). Other conditions, as in Figure 2.

to higher peak-to-peak excitation voltages and synchronous detection, described very recently by the Hauser group,[15] as well as in connection to on-chip preconcentration schemes.[16,17]

The high sensitivity and speed of the microchip contactless conductivity detection system is coupled to a very good reproducibility and stability. For example, a series of eight repetitive injections of a mixture containing 10 ppm MPA, EMPA, IMPA, and PMPA yielded relative standard deviations of 3.8, 4.7, 4.5, and 5.0%, respectively. Such good precision is attributed to the absence of unwanted surface fouling associated with the contactless operation. The precision of migration times of MPA, EMPA, and IMPA within a given set of experiment was very good (RSD < 1% (n = 30)). The day-to-day reproducibility of the migration times was also good (RSD < 3%). Indeed, the long-term stability of the electroosmotic flow in the PMMA microchip device was very satisfactory (RSD < 3%) upon filling the chip with deionized water during storage. The chip-to-chip variation of the EOF was also good (RSD = 6%) when treated in the same way (washing and storing in deionized water). An assay of a 10 ppm MPA and PMPA sample mixture was used for estimating the accuracy.[4] The recovery percentages ranged from 88 to 98% for MPA and 91 to 95% for PMPA.

The suitability of the microchip CE/contacless conductivity detection for measuring low levels of nerve agent breakdown products in the environmental samples is demonstrated in Figure 6. The electrophoregram for a river sample, spiked with 20 ppm (a) MPA, (b) EMPA, and (c) IMPA, is characterized with three well-defined and baseline-resolved peaks (B). The total assay time is ∼130 s. Note, the flat baseline and the absence of background interferences (A). The large background peak (at 36 s) corresponds to common inorganic anions (e.g., Cl^-, NO_3^-, SO_4^{2-}) present in the water sample. Such anions display an overlapping peak due to electrodispersion associated with their relatively high concentration,[18] in a manner consistent with that reported for conventional CE.[5] The minimal sample preparation (filtration and pH adjustment) makes the method suitable for routine field-screening work. Applicability to other matrixes, including groundwater, surface water, and soil extracts, is expected based on the selectivity reported in analogous conductivity detection for con-

(15) Tanyanyiwa, J.; Galliker, B.; Schwarz, M. A.; Hauser, P. C. *Analyst* **2002**, *127*, 214.
(16) Lichtenberg, J.; Verpoorte, E.; de Rooij, N. F. *Electrophoresis* **2001**, *22*, 258.
(17) Lichtenberg, J.; de Rooij, N. F.; Verpoorte, E. *Talanta* **2002**, *56*, 233.
(18) Bakker, D. R. *Capillary Electrophoresis;* Wiley: New York, 1995.

ventional CE systems.[5] The absence of interfering substances in natural water samples indicates a low likelihood of "false positives".

In conclusion, the results presented here clearly demonstrate that the combination of a CE microchip with a contactless conductivity detection results in a promising tool for the monitoring of organophosphonate nerve agent degradation products. The ability to detect major nerve agent breakdown products is advantageous in terms of speed, efficiency, portability, sensitivity, cost, or sample size compared to conventional capillary electrophoresis or ion chromatography systems. Accordingly, microchip-based detection of nerve agent degradation products should have an impact upon the verification of CWA production and destruction and the prevention of terrorist activity. The new field screening/alarm capability should be supplemented with a detailed identification of the breakdown products.[19] The coupling of extremely low-cost PMMA separation chips and easily constructed contactless conductivity detectors holds promise for creating self-contained field-deployable and disposable (single-use) "lab-on-a-chip" systems. Ongoing efforts in these laboratories are aimed at developing a multichannel "counterterrorism" microchip for providing a timely simultaneous detection of different classes of chemical warfare agents and explosive compounds. New "world-to-chip" interfaces are also being explored for the continuous monitoring of nerve agent and explosive substances. Such capabilities, along with the attractive analytical performance, make the new microfluidic device attractive for addressing the needs of various security scenarios.

(19) Tripathi, D. N.; Pandey, K. S.; Bhattacharya, A.; Vaidyanathaswamy, R. *Anal. Chem*. **1992**, *64*, 823.

ACKNOWLEDGMENT

This research was supported by grants from the Department of Justice (MIPT Program), the Office of Naval Research (ONR Award N00014-02-1-0213), and the U.S. EPA. Mention of trade names or commercial products does not constitute endorsement by EPA for use. The authors thank Dr. F. Opekar (Charles University, Czech Republic), for valuable discussions.

Received for review May 2, 2002. Accepted October 2, 2002.

AC025746P