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Alkylphenol Ethoxylate Degradation Products in Land-Applied Sewage Sludge (Biosolids)

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Alkylphenol ethoxylates, widely used in commercial and household detergents in the United States, can degrade during the wastewater treatment process to more toxic, estrogenic, and lipophilic compounds. These include octylphenol (OP), nonylphenols (NPs), nonylphenol monoethoxylates (NP1EOs), and nonylphenol diethoxylates (NP2EOs). These compounds have received considerable attention due to their acute toxicity and ability to disrupt the endocrine system. In Europe, regulations have been established to control their impact on the environment. In this study, biosolids derived from all 11 U.S. wastewater treatment plants examined contained detectable levels of OP, NPs, NP1EOs, and NP2EOs. Nine exceeded the current Danish land application limit (30 mg/kg; sum of NPs, NP1EOs, and NP2EOs) by 6−33×. NPs were the major component, and their concentrations therein ranged from 5.4 to 887 mg/kg (dry weight). OP, reportedly 10−20× more estrogenic than NP, was detected in these same nine biosolids at levels up to 12.6 mg/kg. Three biosolids were also subjected to the U.S. Environmental Protection Agency Toxicity Characteristic Leaching Procedure Method 1311. NPs and NP1EOs were both detected in the leachate; the former at concentrations from 9.4 to 309 μg/L. On the basis of effect levels published in the literature, alkylphenol ethoxylate degradates in U.S. biosolids may cause adverse environmental impacts.

Introduction

Disposal of sewage sludge generated from wastewater treatment is a major problem due to dwindling landfill space and concerns over incineration byproducts. Application of sludge on agricultural fields and rangeland not only is a less expensive option but also widely viewed as an economic way to recycle nutrients and improve soil characteristics. However, sewage sludge may contain mixtures of contaminants, and the potential effects of these may limit its beneficial use. To protect public health and the marine environment from anticipated adverse effects of sewage sludge constituents, the U.S. Congress amended the Marine Protection, Research, and Sanctuaries Act in 1988 to prohibit open ocean dumping. This ban prompted new legislation for sludge disposal, The Standards for the Use or Disposal of Sewage Sludge, Title 40 CFR, Part 503, February 19, 1993 (Part 503 rule). This rule was in part based on results from the National Sewage Sludge Survey (NSSS) completed in 1988 by the United States Environmental Protection Agency (U.S. EPA) (*1*). In this survey, sludges from 176 wastewater treatment plants (WWTPs) were analyzed for pathogens and 411 compounds including heavy metals, pesticides, PAHs, dioxins, and PCBs. Resulting regulations on biosolid application primarily addressed pathogen and metal burdens due in part to the latter's persistence. Since promulgation of the Part 503 rule, the number of biosolids composting projects has doubled with current potential biosolid usage outweighing production by a margin of 47:1 (*2*). In 1998, the U.S. EPA estimated that 60% of the 6.9 million t of biosolids generated was land-applied and predicted that biosolid usage would increase by 40% by the year 2010 (*3*). The NSSS data were revisited in 1995 for possible additions to biosolid regulations. Chlorinated dioxins/dibenzofurans and coplanar PCBs are now proposed for inclusion under the Round Two Sewage Sludge Regulations (*4*). In our study, alkylphenol ethoxylate (APEO) degradation byproducts [octylphenol (OP), nonylphenols (NPs), nonylphenol monoethoxylates (NP1EOs), and nonylphenol diethoxylates (NP2EOs)], potential xenoestrogens that were not included in the 1988 NSSS, were examined in biosolids from 11 WWTPs. These biosolids originated from four geographic regions of the United States (New England, Mid-Atlantic, South-Central and West Coast) (Figure 1) and encompass four popular types of biosolid stabilization processing [composting, lime (alkali) addition, heating, and anaerobic digestion] (Table 1). These stabilization processes are intended to reduce pathogen levels, odor, and water content prior to land application. After stabilization, biosolids that do not exceed the allowable metals levels receive a classification of "A" or "B" (*3*). Class A is reserved for biosolids that also show no detectable levels of pathogens. These biosolids can be used with similar restrictions as conventional fertilizer or soil amendment products and may be distributed directly to the general public. Biosolids that have detectable levels of pathogens and do not exceed the maximum contaminant levels for metals receive a B classification and can be used on agricultural and grazing lands not in direct contact with humans. These require an additional acclimation period, to reduce pathogens, after being land applied (*3*). In 1998, the U.S. EPA estimated that 0.8 million dry ton (MDT) of sewage sludge was disposed of as class A biosolids and 2.8 MDTs as class B (*3*). In addition to analyzing the 11 biosolids for APEO byproducts, the leachabilities of these contaminants from three biosolids (two class A and one class B) were examined to estimate their potential for possible migration following land application.

In 1994, U.S. consumption of APEOs exceeded 250 000 t (*5*). These surfactants are used in detergents, paints, pesticides, textiles, and personal care products. APEOs have been shown to degrade into more toxic and lipophilic compounds in WWTPs (*6*). Although APEO releases are mainly associated with WWTPs, these compounds have also been detected in non-WWTP effluents (*7*). APEOs are biodegraded by a stepwise shortening of the ethoxylate chains, creating a complex mix of compounds including shorter chain ethoxylates, alkylphenoxy carboxylic acids (APECs), and alkylphenols (APs), such as OP and NPs. APECs and longer chain APEOs are quite water-soluble, thus they predominate in WWTP effluent (*6*) but have also been detected in sewage sludge (*6, 8*). OP, NPs, and the shorter chain APEOs have been reported in receiving waters (*6, 9*) but have lower water solubilities and tend to sorb to suspended solids or sediments (*6*). Therefore, they largely associate with sewage sludge.

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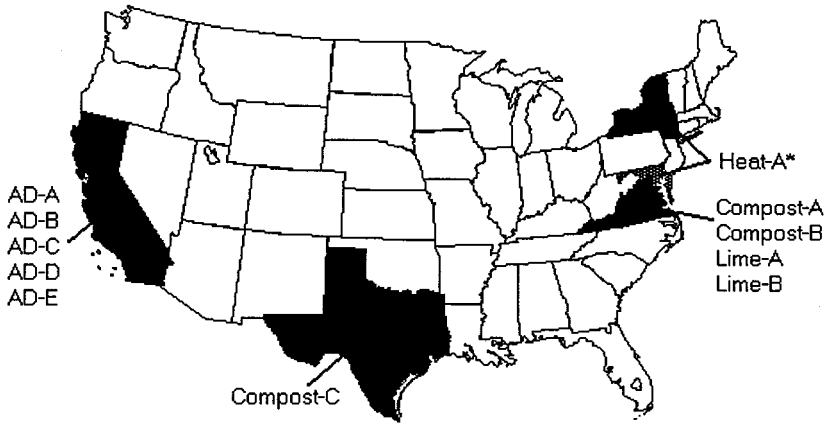

FIGURE 1. Geographic location of biosolid sample sites. (*) Origin of heat A, New York and/or Maryland.

TABLE 1. Biosolid Characteristics

sample	type of stabilization	biosolid classification	% solids	% total organic carbon	% total nitrogen
compost A	compost	A[a]	66	9.9	1.3
compost B	compost	A	45	18.5	2.1
compost C	compost	A[a]	64	16.1	1.6
lime A	lime (alkali)	B	37	12.3	1.6
lime B	lime (alkali)	B	31	24.6	2.9
heat A	heat	A[a]	>95	24.9	4.0
AD A	anaerobic digestion	B	30	23.5	3.5
AD B	anaerobic digestion	B	39	22.2	3.8
AD C	anaerobic digestion	B	34	25.4	4.5
AD D	anaerobic digestion	B	44	20.6	3.5
AD E	anaerobic digestion	B	3.0	28.8	5.2

[a] Biosolids distributed at retail outlets for home garden usage.

Reported log K_{ow} values are 4.12 and 4.48 for OP and NPs and 4.17 and 4.21 for NP1EOs and NP2EOs, respectively (*10*).

Environmental contamination by APEO degradation products has been reported in many areas of the world. Sediment cores taken from areas influenced by WWTPs (including Tokyo Bay, Japan, and the Strait of Georgia, British Columbia, Canada) have shown trends of increasing levels of NPs since the mid-1960s (*11, 12*). These cores also indicate that NPs degrade slowly in anaerobic sediments. Leachate from a Swedish municipal landfill, which received WWTP sludge, was previously shown to contain NPs at 107 μg/L (*13*). In 1989, 30 U.S. rivers expected to contain APEOs were monitored for NPs and NPEOs. This study concluded that over one-third of these rivers contained APEO byproducts. River sediments also contained NPs at up to 3000 μg/kg and NP1EOs at 170 μg/kg, dry weight (*14*). (All values reported on a dry weight basis unless otherwise noted.) Groundwater contamination by APs and APEOs has also been reported in Switzerland, Israel, and the United States (*15*−*17*). OP, NPs, NP1EOs, and NP2EOs have all been detected in drinking water, up to 34 ng/L (*18, 19*). Little research on biodegradation of sludge-bound APEO byproducts in soils has been performed. However, a Canadian study indicated that 60% of the original NPs and 30% of OP remained in the soil 60 days after application but decreased to nondetectable levels 90 days after application (*20*). A Danish study also suggested that soil concentrations of NPs, NP1EOs, and NP2EOs remained constant during a 28-day testing period (*21*).

Recently, concerns have been raised about the potential estrogenic effects of APs. Those with the hydroxyl group in the para position have been shown to displace 17β-estradiol from the estrogen receptor (*22*−*24*). OP, NPs, and NP2EOs have been reported to induce vitellogenin production in male trout and in minnows (*Pimephales promelas*) at low micrograms per liter concentrations (*25, 26*). Expression of intersex (testis-ova) in medaka (*Oryzias latipes*) was also observed following exposure to NPs at 50 μg/L (*27*). Wild roach (*Rutilus rutilus*) associated with discharges from U.K. WWTPs exhibited a high incidence of intersexuality (*28*). Two of these U.K. rivers, the Aire and Lea, were shown to contain xenoestrogens (NPs, NP1EOs, and NP2EOs) at up to 76 μg/L (*29*). These compounds also may bioaccumulate. Both NPs and NP1EOs were detected in fish tissue taken from the Kalamazoo River, Michigan (*30*), and the Tyne and Tees Rivers in the U.K. (*31*). OP was also detected in fish samples from the Tees (*31*). Since 1995, England, France, Germany, and the Scandinavian countries have voluntarily banned APEO use in household cleaning products (*32*). A European Union (EU) initiative regulating biosolids established a 50 mg/kg limit for the total of NPs, NP1EOs, and NP2EOs (*33*). The Danish Ministry of Environment and Energy currently regulates NPs, NP1EOs, and NP2EOs in biosolids. The current cutoff limit is 30 mg/kg, but this will be lowered to 10 mg/kg in 2002 (*34*). Canada has placed NPs and their ethoxylates on their second Priority Substances List (PSL2) and has proposed that they be classified as "toxic", as defined under Section 64 of the Canadian Environmental Protection Act (CEPA).

Currently, there are no U.S. regulations limiting the use or disposal of APEOs or their degradation products. The risk assessment, which serves as the basis for biosolid regulations, is rooted in the results of the 1988 NSSS; however, this survey examined a limited set of analytes. The EPA's 1995 reassessment of Rule 503, the so-called "Round Two", does not represent a new comprehensive examination of contaminants in biosolids. Here the EPA largely re-evaluates the data

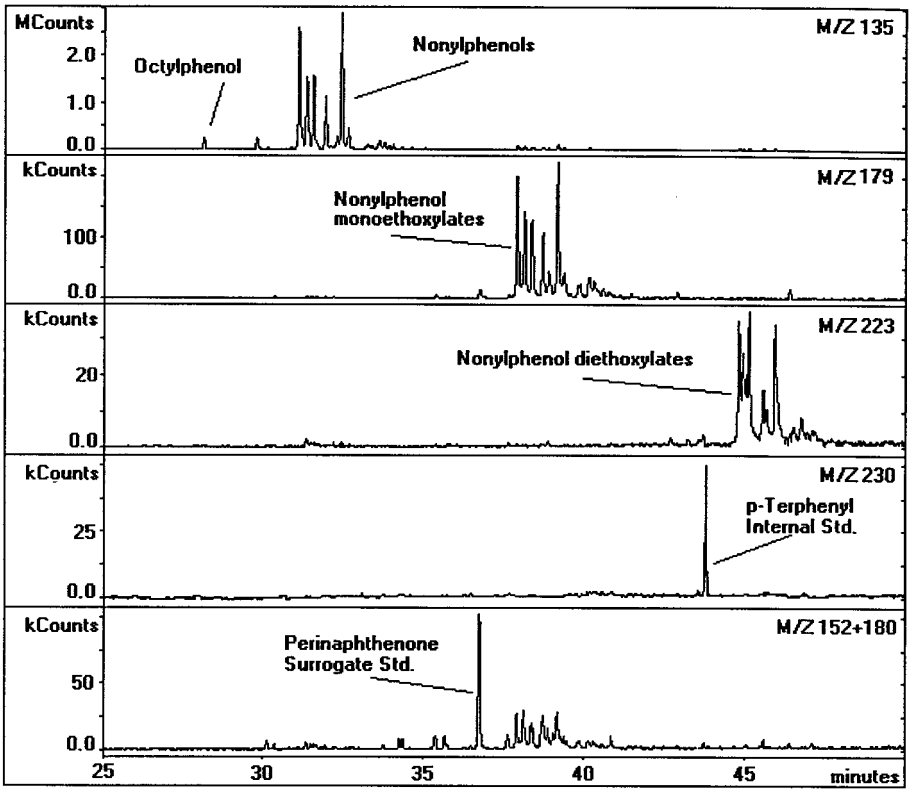

FIGURE 2. GC/MS chromatogram of a biosolid (lime A) extract showing selected characteristic ion peak clusters 135 *m/z* for NPs, 179 *m/z* for NP1EOs, and 233 *m/z* for NP2EOs and single peaks at 135 *m/z* for OP, 230 *m/z* for *p*-terphenyl, and 152 and 180 *m/z* for perinaphthenone.

obtained during the NSSS, conducted over 13 years ago, which does not address increases in chemical consumption or new chemicals entering the market. With concerns multiplying over the protectiveness of Rule 503, the EPA has recently asked the National Academy of Sciences to review the science behind the ruling (*35*). Here, we examine one class of potentially toxic chemicals that may enter the environment through the land application of biosolids.

Experimental Section

In this study, four class A and seven class B biosolids representing four major stabilization techniques were collected prior to land application and analyzed for OP, NPs, NP1EOs, and NP2EOs (Table 1). All samples were freeze-dried, sieved (2000 μm) to remove large debris, and stored in glass jars with Teflon lids at <4 °C until analyzed. Percent solids, total organic carbon (TOC), and total nitrogen (TN) were determined for each (Table 1). Percent solids were determined by heating each sample at 105 °C until a constant weight was established. TOC and TN were analyzed by thermal conductivity detection (Exeter CE440, Chelmsford, MA); inorganic carbon was removed by addition of hydrochloric acid. TN includes inorganic and organic nitrogen. The extraction and purification procedure has been previously described (*7*) and will only be briefly detailed here. For APEO byproduct determination, samples (2−5 g) were subjected to enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA). Conditions were as follows: two extraction cycles, pressure at 1000 psi, temperature at 100 °C, heat 5 min, static 5 min, 60% flush, purge 180 s. Approximately 30 mL of dichloromethane (DCM) were used per sample. Perinaphthenone was added as a surrogate prior to extraction. Extracts were reduced to 5 mL under nitrogen and purified by size exclusion chromatography, (Envirosep-ABC, 350 × 21.1 mm column; Phenomenex, Torrance, CA). The column was eluted with DCM at 5 mL/min. The first 50 mL, containing high molecular weight lipids, were discarded. The next 60 mL, containing the compounds of interest, were collected and solvent exchanged to hexane. The partially purified extract was then added to a 2-g silica column (EnviroPrep, Burdick & Jackson) and eluted with 3 mL of hexane, followed by 6 mL of 60:40 hexane/DCM. OP, NPs, and NPEOs were then eluted with 10 mL of acetone and collected separately. The retained fraction was reduced in volume and solvent exchanged to toluene. *p*-Terphenyl (10 μg) was added as an internal standard prior to gas chromatography (GC).

OP, NPs, NP1EOs, and NP2EOs were separated by GC and identified with a mass spectrometer (Varian Saturn 2000 GC/MS, Sugar Land, TX) operated in the electron ionization (EI) mode, scanning from 50 to 450 *m/z*. Standards were analyzed to determine fragmentation patterns and principal ions. A cluster rather than a single peak, as seen with OP, was observed for NPs, NP1EOs, and NP2EOs (Figure 2) because commercially supplied NPEOs are a mixture of isomers and not a single compound (*29*). Characteristic ions selected were as follows: OP 135 *m/z*, NPs 135 *m/z*, NP1EOs 179 *m/z*, NP2EOs 223 *m/z*, perinaphthenone 152 and 180 *m/z*, and *p*-terphenyl 230 *m/z*; these peak clusters and characteristic ions have been utilized by others (*29*, *36*, *37*). Analytes were quantified with a five-point linear calibration curve bracketing the concentration range of each sample. This was constructed by comparing the internal standard peak area to the sum of the total peak areas of selected characteristic ions for each analyte. The quantification curve was generated with analytical standards: 4-*tert*-octylphenol and 4-nonylphenols (Fluka Chemie AG, Switzerland) and a 60:40 mix of NP1EOs and NP2EOs (ChemService, West Chester, PA). Samples (1.5 μL) were injected, splitless mode, onto a 60 m, DB-5 column (J&W Scientific, Folsom, CA) with a 0.25 μm film thickness and 0.32 mm i.d. The split vent was opened at 0.75 min. Helium carrier gas flow was about 1 mL/min with a head pressure of 15 psi. The GC temperature program used was

TABLE 2. APs and NPEOs (mg/kg, dry weight) in Biosolids

sample	OP (mg/kg)[a]	NPs (mg/kg)[a]	NP1EOs (mg/kg)[a]	NP2EOs (mg/kg)[a]	APs & NPEOs totals
compost A	<0.5	5.4 (5.5)	0.7 (12)	<1.5	6.1
compost B	1.5 (6.1)	172 (4.1)	2.5 (13)	<1.5	176
compost C	<0.5	14.2 (6.6)	<0.5	<1.5	14.2
lime A	5.3 (2.9)	820 (3.0)	81.7 (13)	25.3 (5.0)	932
lime B	2.0 (11)	119 (6.3)	154 (12)	254 (13)	529
heat A	7.5 (3.4)	496 (6.0)	33.5 (12)	7.4 (32)	544
AD A	9.9 (12)	683 (6.1)	28.4 (7.5)	<1.5	721
AD B	12.6 (5.8)	720 (14)	25.7 (6.7)	<1.5	758
AD C	11.0 (7.7)	779 (2.6)	102 (17)	32.6 (9.4)	925
AD D	11.7 (7.3)	701 (9.9)	55.8 (11)	< 1.5	768
AD E	6.7 (1.0)	887 (8.7)	64.9 (20)	22.7 (21)	981
mean, $n = 11$	6.2	491	49.9	31.1	578

[a] % SD is given in parentheses, ($n = 3$).

as follows: initial column setting 75 °C, hold 1 min, ramp at 4 °C/min, hold at 330 °C for 5 min, total run time 70 min, injector 315 °C. The transfer line was set at 320 °C, EI ion source temperature was 250 °C, and emission current was 20 μA. Biosolid quantitation limits were 0.5 mg/kg for OP, NPs, and NP1EOs and 1.5 mg/kg for NP2EOs.

Three biosolids (composts A and B and lime A) were also subjected to the U.S. EPA Toxicity Characteristic Leaching Procedure (TCLP, SW-846, EPA Method 1311) and the leachate analyzed for NPs, NP1EOs, and NP2EOs. (OP analysis was not included in this part of the study.) EPA's Office of Solid Waste has previously utilized this procedure to determine if municipal sewage sludge exceeds toxicity characteristic regulatory levels for pesticides, herbicides, volatile and semivolatile organic compounds, and metals (*38*). The EPA has developed this procedure to simulate leaching from a landfill under a mismanagement scenario (unlined landfill), whereby landfill leachate containing toxicants may enter groundwater**.** The TCLP vessel, a 500-mL glass bottle with Teflon lid, was filled with biosolids and extraction solution at a ratio of 1:20 (dry wt/wt). The suspension was allowed to rotate "end over end" at 30 rpm for 18 h. It was then passed through a 0.7-μm glass fiber filter (No. 66256 Gelman Science, Ann Arbor, MI). Perinaphthenone was added as a surrogate standard to the filtrate (leachate), which was then sequentially extracted in a separatory funnel with three aliquots of DCM, totaling 200 mL. The extract was solvent exchanged to hexane, reduced in volume to <1 mL, and purified by passing through a 2-g silica SPE column as previously described for the biosolids. Each sample was reduced, spiked with the internal standard (*p*-terphenyl), and analyzed for NPs, NP1EOs, and NP2EOs by GC/MS. TCLP quantitation limits were 0.5 μg/L for NPs and NP1EOs and 1.5 μg/L for NP2EOs.

The analytical methodologies for both biosolids and TCLP extracts were investigated by assessing blanks and analyte recoveries of spiked samples. Blanks were analyzed with each set of samples; detected background levels in each blank (n=4) were below the quantitation limit. Compost-A (biosolid) was spiked with 11.3 μg/g NPs, 8.8 μg/g NP1EOs and 5.8 μg/g NP2EOs. Analytical recoveries were 126, 78 and 108%, respectively. OP recoveries were not assessed during this study; however, other researchers have reported OP to behave similarly to NPs in analytical procedure (*29, 37*). The TCLP extraction procedure was assessed by spiking an additional aliquot of Compost-A to final concentrations of 33.8 μg/L NPs, 17.5 μg/L NP1EOs and 11.7 μg/L NP2EOs. Analytical recoveries were 76, 87, and 128%, respectively.

Results and Discussion

Each of the biosolids contained at least one of the APEO degradates of concern. Total OP, NPs, NP1EOs, and NP2EOs ranged from 6.1 to 981 mg/kg (Table 2). Results presented are based on triplicate analysis and corrected for surrogate recovery. Surrogate recoveries ranged from 62 to 110%; mean 97%. The percent standard deviation (% SD) was also determined for each compound; mean 10.4% (Table 2). In 10 of the 11 biosolids, NPs were the most abundant APEO byproduct detected, contributing >84% of the total APs and NPEOs. NP concentrations ranged from 5.4 to 887 mg/kg, with a mean of 491 mg/kg (Table 2). Of the four stabilization treatments, the mean NP concentration in the anaerobically stabilized biosolids (754 mg/kg) was nearly twice that of the heat-treated (496 mg/kg) and limed (470 mg/kg) samples and 12-fold greater than in the composted (64 mg/kg) biosolids. It has been suggested that the abundance of a particular metabolite (APs or APEOs) is dependent on the wastewater treatment process. Concentrations of NPs in sewage sludge have been shown to double following aerobic digestion (*6*) and increase up to 15-fold as a result of anaerobic digestion (*39*). Degradation may occur via sequential loss of ethoxy groups, leading to NP2EOs, NP1EOs, and finally NPs (*6*). This stepwise shortening of the ethoxy chain may also explain our failure to detect NP2EOs in 6 of the 8 anaerobic and composted biosolids (Table 2). In contrast, NP2EOs were observed in each of the lime and heat-treated samples.

Lime B appeared to contain anomalously high proportions of NP1EOs and NP2EOs (77%) related to NPs (23%). In this sample, microbial degradation and resultant losses of the longer ethoxy groups may have been terminated by the high pH of the biosolids (>12) resulting from liming. However, this effect was not observed in lime A. APEO fate may be determined not only by microbial transformation but also by physicochemical processes established during the wastewater treatment process (*15*). Both the TOC and TN levels of sewage sludge contribute to their agricultural value and are listed in Table 1. Partitioning of APEO byproducts into the sludge during the wastewater treatment process is enhanced by the latter's high organic carbon content. Once the biosolids are land applied and pH neutralized (in the case of limed biosolids), microbiological activity may further degrade the higher oligomer APEOs, increasing NP concentrations. This microbiological process may also eventually degrade the biosolid's organic fraction, possibly releasing associated APEO byproducts into the soil. It has been reported that NPs degrade under aerobic conditions (*40*), but breakdown has been observed to be slow or nonexistent under anaerobic environmental conditions, e.g., when incorporated in anaerobic sediments (*11, 12*).

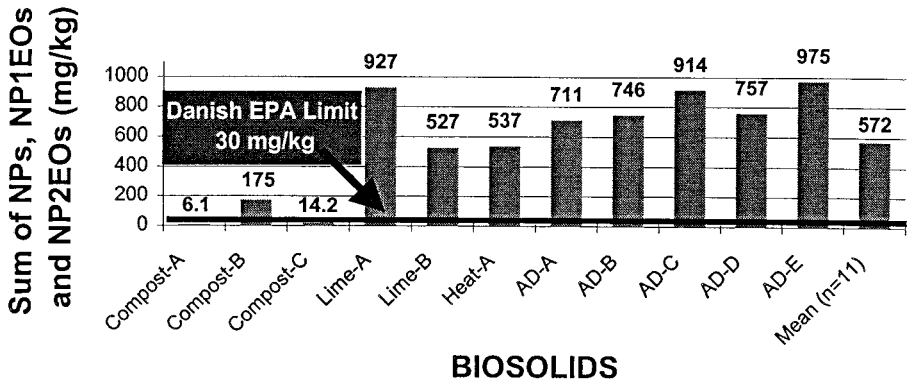

FIGURE 3. Sum of NPs, NP1EOs, and NP2EOs per biosolid as compared to the 1997 Danish EPA application limit (sum of NPs, NP1EOs, and NP2EOs: 30 mg/kg, dry weight). Nine of the biosolids exceeded this limit. (Note: European Union 2000 draft regulation established a 50 mg/kg limit for the sum of NPs, NP1EOs, and NP2EOs; *33*.)

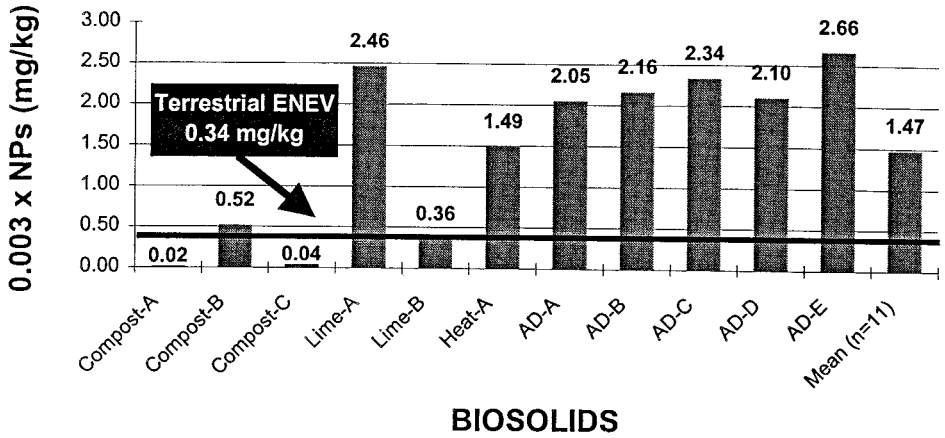

FIGURE 4. After applying the agricultural application rate factor (0.003) to the NP concentrations of the 11 biosolids, 9 were predicted to exceed the Terrestrial NP Estimated No Effects Value (ENEV) of 0.34 mg/kg, as recommended by Environment Canada (*44*).

Of the four stabilization treatments, the composted biosolids had the lowest total APs and NPEOs, ranging from 6.1 to 176 mg/kg (Table 2). The lower values for the compost samples could be a function of the source sludge or the subsequent sludge treatment process. Unlike limed, heat-treated, or anaerobic digested biosolids, composted biosolids are a mixture of sewage sludge and other organic waste, e.g., wood, leaves, and yard waste. This mixture lowers the overall percentage of sludge in the product, thus diluting contaminants therein. Also, compost piles are aerated by mixing (windrow composting) or via blowers connected to perforated pipes or grates running under the piles (aerated static piles). These steps may facilitate further aerobic degradation of APEOs and likely some NPs (*40*) as compared to liming, heating, or anaerobic digestion.

A comprehensive study of NPs, NP1EOs, and NP2EOs levels in sludge from 19 Danish WWTPs was conducted in 1995. Concentrations ranged from 0.3 to 67 mg/kg (*21*). With the exception of the U.S. composted biosolids, the Danish results are about an order of magnitude lower than what we observed in our study of U.S. biosolids. This difference may be due to lower APEO consumption rates in Denmark as a result of voluntary restrictions spurred on by APEO byproduct environmental impact concerns (*32*). The Danish EPA set a 30 mg/kg biosolid land application limit in 2000 for the sum of NPs, NP1EOs, and NP2EOs (*34*). Application of this threshold on U.S. biosolids would result in prohibition of the application of 9 of the 11 biosolids examined (Figure 3). Although the Danish limit is primarily based on the European precautionary principle, the magnitude by which APEO byproducts differ between U.S. and Danish biosolids is striking. Only composts A and C were below the current Danish limit, and compost C exceeds the 10 mg/kg limit proposed for 2002 (*34*).

OP, which is not presently regulated in Europe or the United States was also detected in 82% of the biosolids tested, ranging from <0.5 to 12.6 mg/kg (Table 2). While it was present at concentrations less than 2% of NPs, it has been previously reported that OP is 10−20-fold more estrogenic than NPs or NP2EOs in vitro (*41*). An OP bioconcentration factor (BCF) in rainbow trout (*O. mykiss*) was determined to be 100−260 as compared to 24−98 for NPs (*42*). Thus, the OP concentrations found in U.S. biosolids may be at sufficient levels to merit further evaluation.

Effects of APs and APEO-related compounds on aquatic organisms have been documented (*25*−*27, 43*). Comparatively, little research has been done to examine their effects on terrestrial biota. However, one study with earthworms (*Apporectodea calignosa*) reported a 21-day EC_{50} (reproduction) of 3.44 mg/kg for NPs in soil (*44*), which is also consistent with a 14-day EC_{50} (reproduction) of 16 mg/kg for collembolan (*Folsomia candida*) (*21*). On the basis of the earthworm study, Environment Canada recommended an Estimated No Effects Value (ENEV) for terrestrial risk due to NP exposure of 0.34 mg/kg (*44*). Under current U.S. biosolid agriculture application rates (based on crop nitrogen uptake), it is estimated that an annual application of 3 dry tons of biosolids per acre would be allowed (*4*). Assuming that biosolids are applied and tilled to a depth of 15 cm and 1 acre weighs 1000 ton, a biosolid application factor of 0.003 is estimated. By multiplying our NP results for each of the biosolids by this application factor (0.003), it appears that 9 out of the 11 biosolids analyzed would exceed the Canadian recommended NP ENEV of 0.34 mg/kg (Figure 4). According to U.S. EPA

TABLE 3. NPs and NPEOs (μg/L) in Leachate Derived from Extraction of Three Biosolids Using the U.S. EPA Toxicity Characteristic Leaching Procedure (TCLP)

sample	NPs	NP1EOs	NP2EOs	total of NPs, NP1EOs, and NP2EOs	% of total leached (NPs + NP1EOs + NP2EOs)
compost A	9.4	<0.5	<1.5	9.4	1.8
compost B	196	5.9	<1.5	202	2.3
lime A	309	19.4	<1.5	328	1.2
mean, $n = 3$	172	12.4	<1.5	180	1.8

estimates, yearly biosolid application rates on public contact sites (e.g., parks), forest, and reclamations sites exceed agriculture rates by 2.6, 3.7, and 10.6 times, respectively (*4*), potentially resulting in higher NP burdens in these terrestrial environments.

TCLP leachate from two composted and one lime-stabilized biosolid were analyzed for NPs, NP1EOs, and NP2EOs. The sum of NPs and NP1EOs for the lime A leachate was 328 μg/L, followed by 202 and 9.4 μg/L for composts B and A, respectively (Table 3). Leachate levels of the APEO byproducts were proportional to the amounts in the original material. NPs exceeded NP1EOs and NP2EOs in all cases. Approximately 2% of the total APEO byproduct present in each biosolid was leached (Table 3). While potential concentrations produced by batch extractions, such as the TCLP, differ from leachates generated under natural conditions in the field, a comparison of concentrations to known effects levels may be an instructive "worst-case" exercise. LC_{50} (96-h) values for NPs in 22 different species of fish ranged from 17 to 3000 μg/L, with median values generally between 100 and 300 μg/L (*43*). Low levels of NPs (10 μg/L) have also been reported to stimulate vitellogenin synthesis in male rainbow trout (*O. mykiss)* (*25*). Thus, leachates may have the potential under some conditions to produce deleterious effects in exposed organisms.

Our results indicate that APs and NPEOs are being introduced and distributed in the environment through land application of biosolids, which is not confined to a single geographic region in the United States or a biosolid stabilization process. APEO degradation products can produce toxic and estrogenic effects, indicating that these contaminants may be a concern in both terrestrial environments, where biosolids are being applied, as well as in aquatic systems that may receive runoff. Although composted samples analyzed generally contained the lowest concentration of APEO degradation products, TCLP leachate from these contained NP levels that exceeded known effects thresholds (e.g., vitellogenin production). While studies of environmental contamination of APEO degradation products have primarily focused on surface water, contamination of groundwater through the disposal of APEO-bound sewage effluent in shallow unconfined aquifers has been reported (*45*). APEOs may not be the only biosolid contaminant of concern as previously unrecognized persistent organic pollutants, such as brominated diphenyl ethers (BDEs), a class of flame retardants, have recently been detected in U.S. biosolids at milligrams per kilogram (dry weight) levels (*46*). Cumulative effects of APEO byproducts in these complex mixtures of biosolid pollutants also merit attention as components of binary mixes (e.g., NP and methoxychlor) below LOEC levels (<10 μg/L) have been shown to induce vitellogenin production in rainbow trout (*47*). With continuing efforts to reduce waste by recycling along with a 50% nationwide decrease of landfills between 1988 and 1995 (*2*), traditional means of sewage sludge disposal are being challenged, increasing incentives to land apply. Further research on the distribution of APEO degradation products and their cumulative effects on both terrestrial and aquatic organisms are needed along with continuing efforts to identify additional synthetic organic constituents in biosolids and their ramifications.

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Literature Cited

(1) National Sewage Sludge Survey: Availability of Information and Data, and Anticipated Impacts on Proposed Regulations. *Fed. Regist.* **1990**, November 9, (55 FR 47210-47283).
(2) U.S. Department of Agriculture. *Agricultural Uses of Municipal, Animal, and Industrial Byproducts, Conservation Research*; Report 44; Agricultural Research Service; Gaithersburg, MD, 1998.
(3) U.S. EPA. *Biosolids Generation, Use and Disposal in the United States*; EPA/530/R-99/009; Office of Solid Waste and Emergency Response: Washington, DC, 1999.
(4) U.S. EPA. *Technical Support Document for Round Two Sewage Sludge Pollutants*; EPA/822/R-96/003; Office of Water: Washington, DC, 1996.
(5) U.S. International Trade Commission. *Synthetic Organic Chemicals, U.S. Production and Sales 1994*; USITC Publication 2933; USITC: Washington, DC, 1997.
(6) Ahel, M.; Giger, W.; Koch, M. *Water Res.* **1994**, *5*, 1131−1142.
(7) Hale, R. C.; Smith, C. L.; de Fur, P. O.; Harvey, E.; Bush, E. O.; La Guardia, M. J.; Vadas, G. G. *Environ. Toxicol. Chem.* **2000**, *4*, 946−952.
(8) La Guardia, M. J.; Hale, R. C.; Harvey, E.; Mainor, T. M. *Proc. SETAC* **1999**, *241*, 53.
(9) Bennie, D. T. *Water Qual. Res. J. Can.* **1999**, *34* (*1*), 79−122.
(10) Ahel, M. and Giger, W., *Chemosphere* **1993**, *26* (*8*), 1471−1478.
(11) Yamashita, N.; Kannan, K.; Imagawa, T.; Villeneuve, D. L.; Hashimoto, S.; Miyazaki, A.; Giesy, J. *Environ. Sci. Technol.* **2000**, *34*, 3560−3567.
(12) Shang, D.; MacDonald, R.; Ikonomou, M. G. *Environ. Sci. Technol.* **1999**, *33*, 1366−1372.
(13) Oman, C.; Hynning, P. *Environ. Pollut.* **1993**, *80*, 265−271.
(14) Naylor, C. G.; Mieure, J. P.; Adams, W. J.; Weeks, J. A.; Castaldi, F. J.; Ogle, L. D.; Romano, R. R. *J. Am. Oil Chem. Soc.* **1992**, *69* (*7*), 695−703.
(15) Tamage, S. S. *Environmental and Human Safety of Major Surfactants, Alcohol Ethoxylates and Alkylphenol Ethoxylates*; Lewis Publishers: Chelsea, MI, 1994.
(16) Zoller, U. *Water Sci. Technol.* **1993**, *27*, 187−194.
(17) Ruthann, A. R.; Melly, S. J.; Geno, P. W.; Sun, G.; Brody, J. G. *Environ. Sci. Technol.* **1998**, *32*, 861−869.
(18) Clark, L. B.; Rosen, R. T.; Hartman, T. G.; Louis, J. B.; Suffet, I. H.; Lippincott, R. L.; Rosen, J. D. *Int. J. Environ. Chem.* **1992**, *47*, 167−180.
(19) Kuch, H. M.; Ballschmiter, K. *Environ. Sci. Technol.* **2001**, *35*, 3201−3206.
(20) Environment Canada. *Alkylphenol Ethoxylate Persistence in Biosolids Treated Field*; Soil Project 71773; Environment Canada and National Water Research Institute; 1998.
(21) Danish EPA. *Effects of Organic Chemicals in Sludge Applied to Soil: Degradation and Toxicity to Organisms Living in Soil*; Ministry of Environment and Energy: 1998.
(22) Mueller, G. C.; Kim, U. H. *Endocrinology* **1978**, *102*, 1429−1435.
(23) Soto, A. M.; Sonnenschein, C.; Chung, K. L.; Fernandez, M. F.; Olea, N.; Serrano, F. O. *Environ. Health. Perspect.* **1995**, *103*, 113−122.
(24) Routedge, E. J.; Sumpter, J. P. *J. Biol. Chem.* **1997,** *272*, 3280−3288.
(25) Jobling, S.; Sheahan, D.; Osborne, J. A.; Matthiessen, P.; Sumpter, J. P. *Environ. Toxicol. Chem.* **1996**, *2*, 194−202.

(26) Harries, J. E.; Runnalls, T.; Hill, E.; Harris, C. A.; Sumpter, J. P.; Tyler, C. R. *Environ. Sci. Technol.* **2000**, *34*, 3003−3011.
(27) Gray, M. A.; Metcalfe, C. D. *Environ. Toxicol. Chem.* **1997**, *5*, 1082−1086.
(28) Jobling, S.; Nolan, M.; Tyler, C. R.; Brighty, G.; Sumpter, J. P. *Environ. Sci. Technol.* **1998**, *32*, 2498−2506.
(29) Blackburn, M. A.; Kirby, S. J.; Waldock, M. J. *Mar. Pollut. Bull.* **1999**, *38*, 109−118.
(30) Keith, T. L.; Snyder, S. A.; Naylor, C. G.; Staples, C. A.; Summer, C.; Kannan, K.; Giesy, J. *Environ. Sci. Technol.* **2001**, *35*, 10−13.
(31) Lye, C. M.; Frid, L. J.; Gill, M. E.; Cooper, D. W.; Jones, D. M. *Environ. Sci. Technol.* **1999**, *33*, 1009−1013.
(32) Renner, R. *Environ. Sci. Technol.* **1997**, *31* (*7*), 316A−320A.
(33) EU. *Working Document on Sludge 3rd Draft*; ENV.E.3/LM; Europa European Commission: Belgium, 2000.
(34) Danish EPA. *Statutory Order from the Ministry of Environment and Energy, On Application of Waste products for Agricultural Use*; No. 49; Ministry of Environmental and Energy: 2000.
(35) Renner, R. *Environ. Sci. Technol.* **2000**, *34*, 242A−243A.
(36) Ding, W.; Tzing, S. *J. Chromatogr. A* **1998**, *824*, 79−90.
(37) Ding, W.; Fann, J. *J. Chromatogr. A* **2000**, *866*, 79−85.
(38) U.S. EPA. *Cooperative Testing of Municipal Sewage Sludges by the Toxicity Characteristic Leaching Procedure and Compositional Analysis*; EPA/430/09-91-077; Office of Water: Washington, DC, 1991.
(39) Giger, W.; Ahel, M.; Koch, M.; Laubscher, H. U.; Schaffner, C.; Schneider, J. *Water Sci. Technol.* **1987**, *19*, 449−460.
(40) Naylor, C. G. *Abs. SETAC* **1999**, *240*, 53.
(41) White, R.; Jobling, S.; Hoare, S. A.; Sumpter, J. P.; Parker, M. G. *Endocrinology* **1994**, *1*, 175−182.
(42) Ferreira-Leach A. M. R.; Hill, E. M. *Mar. Environ. Res.* **2001**, *51*, 75−89.
(43) Servos, M. *Water Qual. Res. J. Can.* **1999**, *34* (1), 123−177
(44) Environment Canada. *Priority Substances List Assessment Report Nonylphenol and Its Ethoxylates, Draft for Public Comments*; Environment Canada/Health Canada: 2000.
(45) Barber, L. W.; Thurman, E. M.; Schroeder, M. P. *Environ. Sci. Technol.* **1988**, *22*, 205−211.
(46) Hale, R. C.; La Guardia, M. J.; Harvey, E. P.; Gaylor, M. O.; Mainor, T. M.; Duff, W. H. *Nature* **2001**, *412*, 140−141.
(47) Thorpe, K. L.; Hutchinson, T. H.; Hetheridge, M. J.; Scholze, M.; Sumpter, J. P.; Tyler, C. R. *Environ. Sci. Technol.* **2001**, *35*, 2476−2481.

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