Environmental Science and Pollution Research

International

Official Organ of the EuCheMS Division for Chemistry and the Environment (EuCheMS DCE)

Editor-in-Chief: Otto Hutzinger

The Rocky Mountain Arsenal, northeast of Denver, Colorado, remediated and developed into a wildlife refuge.

ecomed Springer Vol. 12 No. 4 July 2005

Research Articles

Distribution of PCDDs and PCDFs in Soils Collected from the Denver Front Range

Principal Components Analysis of Diffuse Dioxin Sources

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DOI: http://dx.doi.org/10.1065/espr2005.06.271

Abstract

Background, Aims and Scope. The Rocky Mountain Arsenal (RMA) is a US Army facility located northeast of Denver, Colorado that has been listed on the National Priorities List (NPL). It is currently being re-mediated under the authority of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) and the Superfund Amendments and Reauthorization Act of 1986 (SARA). As part of the remediation activities at RMA, indications were found that a source of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) had existed on the RMA. As a result, investigations were undertaken to assess the possible nature and extent of any potential sources of PCDDs and PCDFs on the RMA site. In addition, other studies were conducted that examined PCDD/PCDF contamination in the Denver area. The goal of these studies was to examine nature and extent of PCDD/PCDF contamination both on the RMA as well as in the surrounding Denver area. The intent of this study was to characterize sources of dioxins (PCDDs) and dibenzofurans (PCDFs) at low environmental concentrations which might have originated from diffuse sources in the Denver Colorado area and in particular, the Rocky Mountain Arsenal (RMA) using Principal Component Analysis (PCA).

Methods. Over 200 soil samples were collected from the RMA and the Denver area. From the RMA, soil was collected as part of three studies that included a (1) random site-wide sampling of the RMA, (2) soils from the Western Tier Parcel (WTP), and (3) soils from Historic Use areas. Denver area soil samples were collected from five different land use categories: Residential, Agricultural, Open Space, Commercial, and Industrial. PCA was conducted on concentrations of 17 2,3,7,8-substituted PCDD and PCDF congeners in 220 soil samples collected from the RMA and the Denver Front Range region.

Results and Discussion. PCA demonstrated the presence of possible minor sources of dioxins on the RMA. Current remediation efforts on RMA will result in the removal of these sources. Samples from the RMA were identified by the presence of a congener profile containing higher chlorinated PCDFs while the Denver Front Range areas were characterized by the presence of higher chlorinated PCDD congeners. The presence of a PCDF signature for the RMA samples does not necessarily indicate a major source of these contaminants on-site. Indeed, the relatively diffuse nature of the sample clusters would argue strongly against the presence of a single large source. Instead, the predominance of the PCDF congeners probably indicates the mixed industrial activities that took place on and near the site.

Conclusion. PCA results indicate that PCDD/PCDF profiles in soils collected from the RMA differed from those collected from the outlying Denver areas but that a major source of these contaminants was not present. Rather, the diffuse nature of sample clusters from the PCA indicated that the congener profile of RMA samples was most likely a result of the mixed industrial activities that historically have taken place on and near the site. PCA also indicated that many of the 'open area' (peripheral site-wide) RMA soils samples did not differ from Denver are reference congener profiles. This finding was also true for samples collected from the WTP that were essentially indistinguishable from Off-RMA reference samples. In addition, total TEQ concentrations in soils collected from WTP were similar to those measured in soils collected from the Denver Front Range areas indicating that lack of a major source of PCDD/PCDF within the WTP zones of the RMA.

Recommendation and Outlook. Analytical as well as statistical results of the soil congener data indicate that the WTP soils are indistinguishable from soils collected from non-industrial areas in the Denver area. This finding would support the recent 'delisting' of the WTP from the other RMA areas and its transfer to other authorities in the Denver area.

Keywords: Diffuse dioxin; distribution of PCDDs and PCDFs in soils; national priorities list (NPL); polychlorinated dibenzo-p-dioxins (PCDDs); polychlorinated dibenzofurans (PCDFs); principal component analysis (PCA); Rocky Mountain Arsenal (RMA); soils; superfund amendments and reauthorization act (SARA)

Introduction

The Rocky Mountain Arsenal (RMA) is a 70 km^2 US Army facility located northeast of Denver, Colorado (**Fig. 1**). The RMA was established in 1942 during World War II to manufacture war materials (Banton et al. 1996). The manufacturing plants and associated facilities were located in the center of the site, surrounded by buffer zones. Following World War II, portions of RMA were leased to private industry,

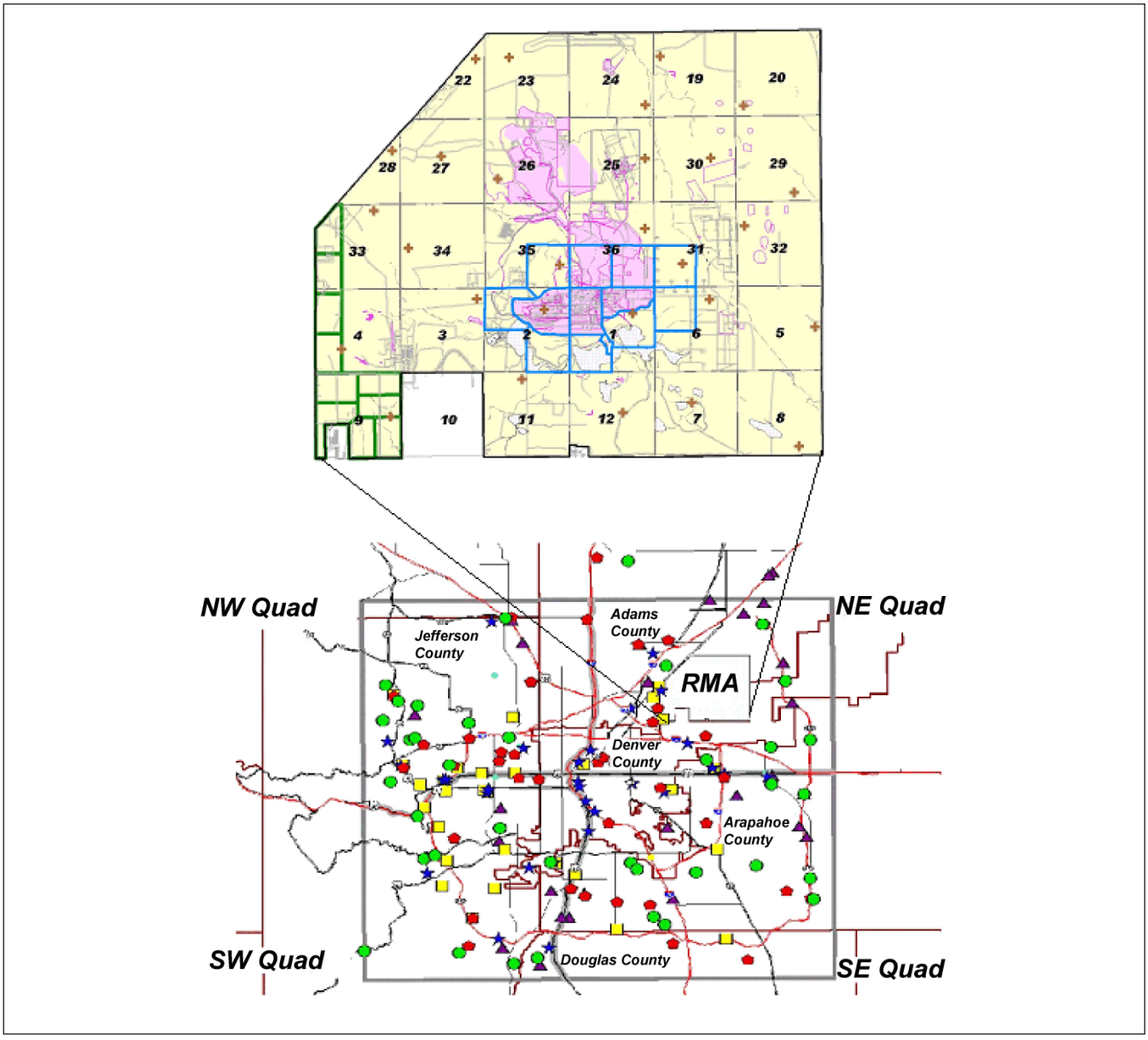

Fig. 1: Sampling locations on the Rocky Mountain Arsenal (RMA) and along the Denver Front Range. Symbols on the lower map indicate locations of sampling in the greater Denver area (Triangles = agricultural; squares = commercial; stars = industrial; circles = open space; pentagons = residential). Symbols on the upper map indicate where samples were collected for the 'site wide' sampling. Historic use samples were collected in the South Plants area (blue squares); WTP samples were collected in the WTP area (green squares)

primarily for the production of pesticides including the manufacture of the chlorinated pesticides aldrin, dieldrin, and chlordane. Organophosphate insecticides, carbamate insecticides, herbicides, and soil fumigants were also produced on the site. Chemical by-products from these activities were introduced into the environment primarily through the burial or surface disposal of solid wastes, discharge of wastewater to unlined or asphalt-lined basins, and leakage of wastewater and industrial effluents through demilitarization activities, routine application of pesticides, and accidental chemical spills and releases. In 1987, RMA was placed on the National Priorities List (NPL) and is currently being re-mediated under the authority of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) and the Superfund Amendments and Reauthorization Act of 1986 (SARA). During remediation activities at RMA, principally to remove organochlorine pesticide contamination, indications were found that a source of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) had existed on the RMA. As a result, investigations were undertaken to assess the possible nature and extent of any potential sources of PCDDs and PCDFs and to determine whether additional specific activities would be required to remediate possible PCDD/PCDF contamination. One study examined the contamination of PCDD/PCDFs in the tissues of American kestrels (*Falco spar-*

verius), common carp (*Cyprinus carpio*) and great horned owls (*Bubo virginianus*) collected from the RMA and from offsite (Coady et al. 2001). In addition, a second set of studies was undertaken to evaluate PCDD/PCDF concentrations in soils as part of a series of opportunistic and targeted monitoring programs from locations within the RMA and in the Denver Front Range areas surrounding area. The goal of these studies was to evaluate the nature and extent of PCDD/PCDF contamination within the RMA and in the surrounding area and to identify potential sources of these contaminants. An issue of particular importance was whether certain areas of the RMA could be 'de-listed' and used for residential or commercial projects. However, analyses had not yet been conducted that examine the nature of PCDD/PCDF congener distributions at these locations relative to potential de-listing of certain RMA areas including the Western Tier Parcel. The objectives of this study were to: (1) Evaluate the differences in TCDD-Toxicity Equivalents in soils from the RMA and in the surrounding Denver area, (2) Evaluate PCDD/PCDF congener patterns in soils from the RMA site, (3) Evaluate PCDD/PCDF congener patterns in soils from the Denver Area, and (4) Compare and contrast PCDD/PCDF congener patterns in soils collected from both the RMA and the Denver Front Range area.

The use of pattern recognition techniques and particularly Principal Components Analysis (PCA) has been widely applied to the analysis of multi-component chemical contaminant mixtures (Meglen 1992, Wenning et al. 1993, Wenning et al. 1999). In particular this type of analysis has proven useful in the identification and apportionment of multiple sources of multi-component mixtures such as PCBs and dioxins (Dunn et al. 1984, Erickson 1997). These chemical groups are particularly amenable to the use of PCA because they consist of well defined mixtures of a large (~200 components) but finite number of individual components. In addition, the source mixtures of these chemicals have relatively distinct and well-defined chemical profiles (Manninen et al. 1996). While the source apportionment of combinations of technical mixtures can be relatively easily achieved using pattern recognition techniques or multiple linear regression (Rappe 1994, Ikonomou et al. 2002), there has been more variable success in the analysis of environmentally 'weathered' or sorted mixtures.

1 Materials and Methods

1.1 Sampling areas

1.1.1 Denver Front Range soil samples

The area selected for investigation encompassed the Denver Front Range area that is defined as a square that is approximately 48 km on a side, centered approximately in Denver, Colorado (see Fig. 1). This area encompasses approximately 2600 km^2, and includes a variety of land uses. All soil sample locations in this study were on governmental (public) lands, including properties controlled by Federal, State, County, or other regional agencies. The study area was divided into four quadrants, and efforts were made to distribute sampling locations evenly between the quadrants. Initially, five different land use categories were considered:

Residential – Land within 60 m of, or adjacent to, residential development, but not within private yards. This included public parks, neighborhood greenbelts and trails, and street medians. Schools and playgrounds were not included in this category.

Agricultural – Land currently, or had been within the past 40–50 yr, tilled and used for crop production.

Open space – Land greater than 0.08 km^2 in area that has not been developed or improved, land essentially in its natural state with the exception of minor changes. Modifications include hiking trails or dirt access roads and some lands used for grazing of livestock.

Commercial – Land developed and used for commercial purposes, such as shopping centers, restaurants, office buildings, RMA offices, etc.

Industrial – Land used for manufacturing, refining, warehousing, or transportation purposes (e.g., garages, railroads, etc.). Approximately 30 samples were collected from each of the different land uses, for a total of 150 samples (USEPA 2001a). Samples were distributed across the study area (see Fig. 1), helping to ensure that the data were representative of the local area. Soil samples were not collected from locations that were known to have been covered with fill or used for 'borrow' material within the last 10 years, since the dioxin content of such recently disturbed areas might not be representative of surrounding undisturbed background areas.

1.1.2 RMA 'site wide' soil samples

The RMA was divided into 28 Sections and one grab sample was collected from the center of each section at a randomly selected location (see Fig. 1) (USEPA 2001b).

1.1.3 Western tier parcel soil samples (WTP)

The Western Tier Parcel (WTP) is a special administrative area of the RMA that has been designated to be transferred to the authority of the neighboring Commerce City. For the purpose of evaluating the potential health risks from dioxins in surface soils, the WTP was subdivided into 10 sub-parcels of approximately 0.4 km^2 each (see Fig. 1). Within each sub-parcel, sets of five surface soil samples were collected using a stratified random sampling scheme to ensure spatial representativeness of the samples (USEPA 2001c). Exact sampling locations were selected where soil appeared to be undisturbed and that were judged to be 'characteristic' of the sub-parcel.

1.1.4 Historic use samples

The area of chief potential concern on RMA was the South Plants area, located in the south-center of the site (USEPA 2001d). In the past, this area was the chief location of pesticide and chemical manufacturing activities. In order to plan the collection of samples in this area, a 12-section grid was laid out over the South Plants area. Within each grid, sets of five grab samples were collected from random sampling locations (see Fig. 1). These five grab samples were combined

into a single composite sample – one for each grid. In addition to South Plants, there are a number of other areas at RMA where historic land uses or waste disposal activities might have resulted in increased levels of dioxins in soil. One composite sample (prepared from five randomly located grab samples, as described above) were collected from each of these 10 'Purposeful' sampling locations

1.2 Sample collection and storage

Soil samples collected for this study were grab samples collected at 0–5 cm in depth. Samples were collected using clean techniques that included use of disposable stainless steel trowels and plastic gloves. A ruler was used to ensure that the actual depth to which soil was collected was within 1.25 cm of the target depth. Loose debris and most gravel or pebbles were removed from the sampling site. The surface soil was placed directly into a clean 16-ounce amber glass jar, filled to capacity (approximately 500 g soil), sealed with a teflon-lined lid. Samples were stored in these bottles at room temperature in the dark until shipped for preparation and analysis. Samples were air-dried and weighed, followed by coarse-sieving through a 2 mm stainless steel screen. The fraction passing the coarse screen was referred to as the 'bulk' fraction. A potion of the bulk fraction was stored for possible future use. The remainder of the bulk fraction was sieved through a 250 µm stainless steel screen to isolate the 'fine' fraction. The fine fraction was thoroughly mixed and placed in sample bottles for storage and shipping.

1.3 Sample analysis

Samples were submitted to Midwest Research Institute (Kansas City, MO) for congener specific PCDD/F analysis. Congener concentrations were determined using an isotope dilution method with high-resolution gas chromatography/mass spectrometry (HRGC/HRMS). Samples were fortified with known quantities of ^{13}C-labeled PCDD/PCDF before extraction with organic solvents and clean-up using column chromatography procedures. Detailed analytical methods are provided elsewhere (USEPA 1999). The Method Detection Limit (MDL) for the study-specific analytical method was defined as an analyte signal that was 2.5 times the average background signal. MDL values varied among samples and among analytes. The Method Quantitation Limit (MQL) was based partly on the lowest calibration standard used, and was defined as a signal that was 10-times the average signal/noise. Because the noise level varied among samples and analytes, MDLs and MQLs also varied. MQL values for most PCDD/PCDF congeners were between 0.5 and 2.5 ng/kg dry weight. The final data set consisted of concentration data 220 soil samples. Values less than MDL were set to half the MDL and other flag-specific adjustments were applied (Slymen and de Peyster 1994).

1.4 TCDD-equivalents in soil

TCDD-equivalents (TEQ) in soils were calculated as the TEF-weighted sum of each of the 17 PCDD and PCDF congeners quantified in soil:

$$TEQ_{soil} = \sum_{i=1}^{17} (C_i * TEF_i)$$

Where TEQs are measured in soils are in units of ng TEQ/kg dry weight, TEF is toxicity equivalency factor for congener$_i$ (van den Berg 1998), C_i is the concentration of congener$_i$ in soil as ng/kg dry weight. For samples where congener concentrations were less than their method detection limit (MDL), half the MDL was used to calculate the TEQ.

1.5 Statistical analysis

All statistical procedures were conducted using SYSTAT Version 10 (SPSS Inc., Chicago, IL). Data sets were tested for normality using visual assessment of probability plots and using a one sample Kolmogorov-Smirnov Test (Filliben 1975, Looney and Gulledge 1985). For these tests samples were grouped by land-use type. Significantly non-normal data was log-transformed and re-tested for normality. Distributions of TEQ values for each land use category were compared using Kruskal-Wallis one way analysis of variance (ANOVA) using ranks. Multiple pair-wise comparisons of TEQs were made with Mann-Whitney Rank Sum tests. Prior to conducting the Principal Component Analysis (PCA), soil congener data were scaled (converted to 'Z' scores, mean = 0 s.d. =1) to minimize bias associated with the order of magnitude differences in congener concentrations (Schwartz and Stalling 1991, de Wit et al. 1992). Principal component analysis was performed with log-transformed data, using the correlation matrix with pair-wise deletion and 'Varimax' rotation (Jackson 1991).

2 Results and Discussion

2.1 TEQs in soil

Soil TEQ concentration in samples from the Denver Front Range varied from a minimum of less than 0.1 ng TEQ/kg up to a maximum of 155 ng TEQ/kg dry weight (**Table 1**). While most of the TEQ values were relatively low, samples collected from land uses categorized as Agricultural or Open Space tended to have TEQ values that were lower than those measured from Commercial or Industrial areas. Multiple pair-wise comparisons of the TEQ data indicated that land use data was separated into two groups. The first group included Open Space and Agricultural lands while the second group consisted of Industrial, Commercial, and Residential data. While TEQ concentrations based on land used did not differ with these two groups, TEQ concentrations between these two groups were statistically different ($p < 0.01$). A comparison of TEQ concentrations between these two groups indicated that average TEQ concentration for the Agricultural and Open Space samples was 1.6 ± 2.0 ng TEQ/kg dry weight while for the Commercial, Industrial and Residential samples the average was 7.7 ± 11.8 ng TEQ/kg dry weight. These values are generally similar to TEQ concentrations measured in soils from rural and urban areas reported in other studies (USEPA 2001a). To evaluate the contribution of congeners that were below their quantification limit relative to the calculated TEQs, a second calculation of TEQs was performed only using congeners that were greater than the method quantification limit (MQL). For

Table 1: Average concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxins equivalents (TEQs) in soils from the Rocky Mountain Arsenal and Denver Colorado area [a]

Land Use	Sample Size (N)	TEQ (ng/kg, dw; ppt)	
		Mean	Range
On-Site (RMA)			
Historic Use	22	12.4	1.2–93.6
RMA Site Wide	28	2.04	0.14–25.4
WTP	10	2.17	1.0–7.2
Off-Site (Denver area)			
Open Spaces	37	1.6	0.1–9.1
Agricultural	27	1.6	0.1–7.7
Residential [b]	37	7.1	0.2–43
Commercial [c]	30	6.4	0.4–57
Industrial	29	9.8	0.2–54

[a] TEQs based on 17 PCDD and PCDF congeners measured in soils, reported on a dry weight basis. TEQs includes congeners concentrations less than the detection limit (< LOQ). These were assigned a value of ½ the LOQ
[b] One outlier value (155 ng TEQ/kg, dw) excluded (see USEPA 2001a)
[c] One outlier value (140 ng TEQ/kg, dw) excluded (see USEPA 2001a)

Off-RMA samples, the average contribution of congeners that were below the MQL was 19% of the total TEQ. For On-RMA samples the average contribution to the total TEQ was 28%, 25%, and 36% for Historical Use, Random Site-Wide, and WTP samples, respectively. Thus, while the contribution of congeners below the MQL may introduce some uncertainty into the calculation of the TEQ values, this would not be expected to significantly alter the overall conclusions of statistical analyses that evaluate the differences of soil TEQ values between the different land use areas.

TEQ concentrations in soil collected On-RMA ranged from 0.14 to approximately 94 ng TEQ/kg dry weight with concentrations from the RMA Historic Use areas being somewhat greater than either the Random Site-Wide or WTP soil samples (see Table 1). Pair-wise comparisons with the Historical Use data showed that South Plants values were not statistically different from Off-RMA industrial areas (p=0.258) but were greater than the other Off-RMA and On-RMA areas (p <0.05). Purposeful samples were greater than the On-RMA random site wide samples and Off-RMA agricultural and open space samples (p <0.01) but did not differ from Off-RMA industrial (p=0.923), commercial (p=0.109) or residential (p=0.247) land use areas. In contrast, On-RMA site wide samples did not differ from Off-RMA open space (p=0.53) and agricultural (p=0.900) lands but were statistically less than samples collected from the Off-RMA commercial, industrial, and residential areas (p <0.01). Overall, these data indicate that except for areas in the immediate vicinity of the former manufacturing areas at RMA, there was no detectable release of dioxins to RMA soils from a site-specific source. Furthermore, it is important to note that the TEQ concentrations in the soils from the Off-RMA locations are far below the USEPA established default concentration value of 1,000 ng TEQ/kg dry weight in surface soils based on human health concerns (USPEA 1998). In addition, of the samples classified as residential only one exceeded the ATSDR 'screening level' of 50 ng TEQ/kg and none approached the ATSDR 'action level' of 1000 ng TEQ/kg (De Rosa et al. 1997).

An evaluation of PCDD/PCDF TEQ congener patterns by land use indicated that there were differences between On-RMA and Off-RMA soil samples (**Fig. 2**). For Off-site RMA land use categories, the dominant congeners based on TEQ

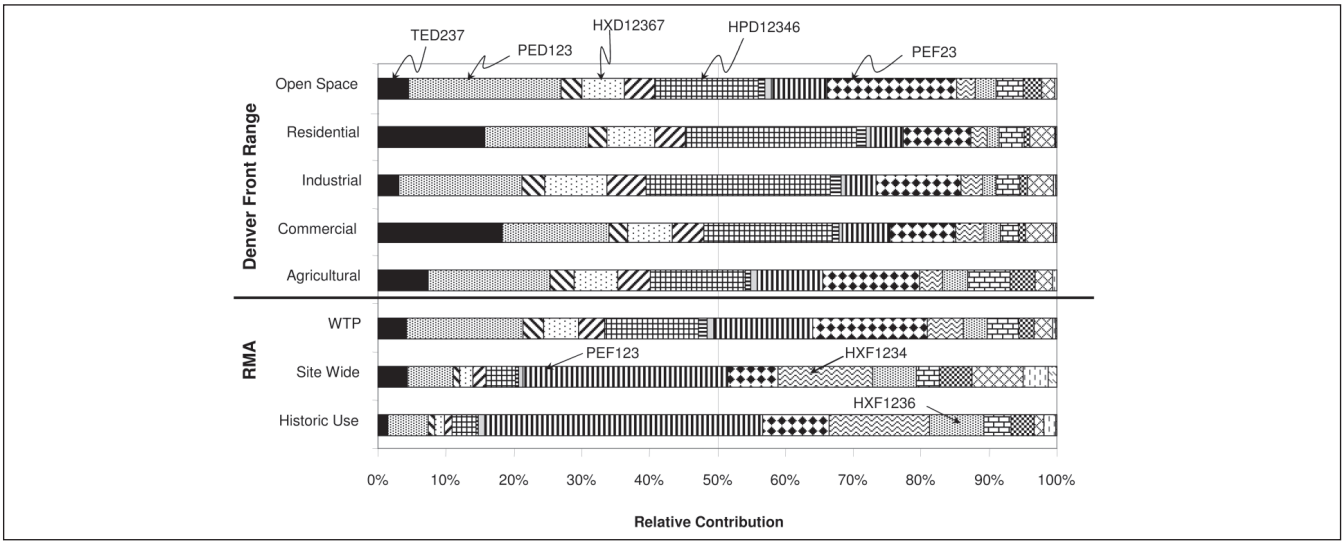

Fig. 2: Contribution of individual PCDD/PCDF congeners to total TEQs in soils collected from the Rocky Mountain Arsenal (RMA) and the Denver Front Range

concentration were 2,3,7,8-TeD (~3–18% of total), 1,2,3,7,8-PeD (~15–18% of total), 1,2,3,4,6,7,8-HpD (~14–27% of total), and 2,3,4,7,8-PeF (~10–19% of total). For WTP samples collected On-site, the relative contribution to these congeners to the total TEQ in soils was similar to that observed for samples collected from areas in the Denver Front Range. This would indicate that the sources of PCDD/PCDFs in these areas are similar. In contrast, the dominant congeners from the Historic Use areas were 1,2,3,7,8-PeF (~41%), 1,2,3,4,7,8-HxF (~15%), and 1,2,3,6,7,8-HxF (~8%). This pattern was also observed in samples collected as part of the Site wide RMA samples indicating that soils from these areas shared a similar TEQ congener pattern that differed from that observed in the Denver area and Western Tier Parcel. However, additional analyses are needed to better define the patterns of PCDD/PCDF congeners between the land use categories.

2.2 PCA of Off-RMA samples

Mean concentrations of 17 2,3,7,8-substituted PCDD and PCDF congeners in soils collected from the Denver Front Range areas ranged from 219 to 2110 ng/kg dry weight (**Table 2**). An evaluation of the untransformed data indicated that concentrations of many of the PCDD/PCDF congeners were not normally distributed. After log transformation, most of the distributions more closely approximated a normal distribution except for congeners that had a significant number of results < MDL such as 2,3,7,8-TCDD. This was expected since the distribution of these congeners was essentially truncated at the MDL and resulted in the accumulation or 'stacking' of values at the MDL or MDL proxy value. Thus, for industrial land-use samples, the distributions of all but one congener were lognormal since very few values were < MDL whereas for the open space samples most of the congeners were not normally distributed due to the greater incidence of < MDL values. Of the congeners evaluated at each location, only 2,3,7,8-TeD was not lognormal due to the large number of < MDL values.

Results from the PCA analysis showed that approximately 87% of the variation in the Off-RMA dataset were accounted for by the first three Factors. Of this variation, Factor 1 accounted for approximately 41% of the variation while Factors 2 and 3 accounted for approximately 35% and 11% of the variation, respectively. 'Loading' values calculated during the PCA were used to examine the contribution of the congeners to each of the Factors. The magnitude of the loading values for each congener are indicative of the significance to the final Factor value and can be used to identify potential congener patterns for each of the designated land use areas. The most significant loadings (r >0.70) to Factor 1 were associated with the more highly chlorinated PCDD and PCDF congeners including 1,2,3,4,7,8-HxD, 1,2,3,7,8,9-HxD, 1,2,3,6,7,8-HxD, 1,2,3,4,6,7,8-HpD, OCDD, 1,2,3,4,6,7,8-HpF, and OCDF. In contrast, the most significant loadings to Factor 2 were from chlorinated PCDF congeners including 2,3,4,7,8-PeF, 1,2,3,7,8-PeF, 1,2,3,7,8,9-HxF, and 2,3,4,6,7,8-HxF. The only congeners that were relatively well segregated into Factor 3 were 2,3,7,8-TeD and 2,3,7,8-TeF. However, only TED2378 had a loading value greater than 0.7 while 2,3,7,8-TeF had a value of 0.518 indicating that while 2,3,7,8-TeD was strongly associated with this factor than 2,3,7,8-TeF.

A scatter plot of PCA results revealed a relatively diffuse grouping of Off-RMA samples with the presence of numerous outliers (**Fig. 3a**). The magnitude of differences of these 'outliers' was evaluated by plotting a sample confidence ellipse based on each of the land-use types (Gnanadesikav and Kettenring, 1972). Any sample lying outside the ellipse is greater than 2 standard deviations from the land-use means for each of the respective factors. Based on this analysis, approximately 20% of Off-RMA samples are 'outliers' from their respective land use groups (31 of 160 samples). Such a relatively great number of 'outliers' suggests that the data sets are relatively 'diffuse' with no strong land-use specific congener profiles. The close overlap of the sample centroids

Table 2: Concentration of PCDD/PCDF congeners in soils collected from the Rocky Mountain Arsenal study area [a]

Congener	On-Site RMA			Off-Site RMA (Denver Front Range)				
	Historic Use	Site Wide	WTP	Agricultural	Commercial	Industrial	Open Space	Residential
2,3,7,8-TeD	0.29 ± 0.33	0.13 ± 3.0	0.11 ± 0.037	0.13 ± 0.32	1.2 ± 3.8	0.32 ± 0.45	0.08 ± 0.06	1.2 ± 3.7
1,2,3,7,8-PeD	1.2 ± 1.7	0.20 ± 0.20	0.43 ± 0.33	0.32 ± 0.30	1.1 ± 1.6	1.9 ± 2.9	0.39 ± 0.43	1.1 ± 1.3
1,2,3,4,7,8-HxD	1.7 ± 2.7	0.29 ± 0.25	0.75 ± 0.56	0.64 ± 1.0	1.8 ± 3.3	3.5 ± 5.6	0.52 ± 0.60	2.1 ± 3.0
1,2,3,6,7,8-HxD	3.0 ± 4.4	0.58 ± 0.55	1.3 ± 0.80	1.2 ± 1.7	4.5 ± 9.0	9.3 ± 17	1.1 ± 1.9	5.2 ± 7.7
1,2,3,7,8,9-HxD	2.2 ± 3.0	0.56 ± 0.52	0.98 ± 0.45	0.84 ± 1.2	3.1 ± 5.7	6.1 ± 10	0.78 ± 1.2	3.3 ± 4.7
1,2,3,4,6,7,8-HpD	70 ± 120	13 ± 16	34 ± 21	25 ± 44	130 ± 280	280 ± 500	27 ± 56	190 ± 380
OCDD	480 ± 830	110 ± 150	330 ± 160	170 ± 220	670 ± 1040	1600 ± 2100	171 ± 280	1100 ± 1700
2,3,7,8-TeF	1.9 ± 2.5	0.19 ± 0.20	0.27 ± 0.13	0.16 ± 0.22	0.39 ± 0.87	0.66 ± 1.6	0.15 ± 0.19	0.46 ± 1.2
1,2,3,7,8-PeF	16 ± 30	1.8 ± 4.4	0.73 ± 0.93	0.35 ± 0.71	0.95 ± 2.4	0.92 ± 1.2	0.29 ± 0.32	0.71 ± 1.2
2,3,4,7,8-PeF	3.8 ± 5.8	0.43 ± 0.62	0.84 ± 0.96	0.51 ± 0.64	1.3 ± 2.8	2.6 ± 3.5	0.67 ± 1.8	1.5 ± 2.7
1,2,3,4,7,8-HxF	29 ± 54	4.1 ± 13.0	1.3 ± 1.7	0.62 ± 1.1	2.9 ± 9.5	3.3 ± 4.4	0.49 ± 0.80	1.8 ± 2.2
1,2,3,6,7,8-HxF	16 ± 28	1.9 ± 5.1	0.90 ± 1.1	0.64 ± 0.95	1.6 ± 3.7	1.9 ± 2.2	0.54 ± 0.89	1.3 ± 1.5
1,2,3,7,8,9-HxF	6.7 ± 13	1.4 ± 4.1	0.59 ± 0.54	0.66 ± 1.1	0.70 ± 0.75	1.1 ± 1.1	0.48 ± 0.44	0.69 ± 0.58
2,3,4,6,7,8-HxF	7.8 ± 13	0.99 ± 2.3	1.1 ± 1.3	1.1 ± 2.7	1.9 ± 3.0	3.7 ± 5.1	0.70 ± 1.2	2.8 ± 6.1
1,2,3,4,6,7,8-HpF	30 ± 36	22 ± 86	6.6 ± 15	4.8 ± 5.7	29 ± 73	40 ± 83	3.2 ± 4.9	27 ± 44
1,2,3,4,7,8,9-HpF	32 ± 66	11 ± 47	1.4 ± 2.3	0.90 ± 2.6	2.5 ± 5.3	3.4 ± 5.7	0.47 ± 0.57	1.9 ± 2.6
OCDF	440 ± 700	370 ± 1800	40 ± 50	12 ± 13	62 ± 140	150 ± 270	9.1 ± 0.8	85 ± 160
Sum [b]	**1140**	**540**	**416**	**219**	**917**	**2110**	**220**	**1430**

[a] Congener data presented as means and standard deviations. All soil concentration data given as ng/kg on a dry weight basis
[b] Values are based on the sum of the average concentration for each congener measured in soil. For congeners <LOQ, ½ MDL was used to calculate the sum

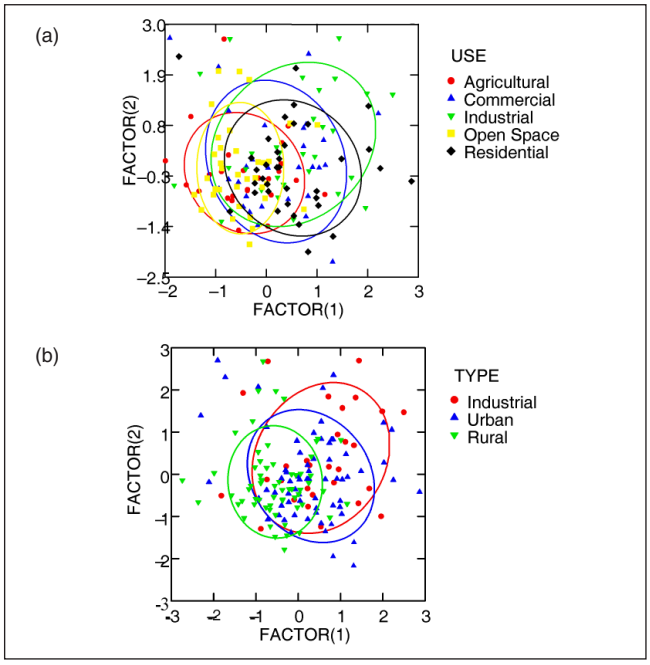

Fig 3: Principal Component Analysis (PCA) of Off-RMA soil samples. (a) PCA results with original land use identifiers. (b) PCA results with modified land use identifiers. Principal Component (Factor) 2 is plotted against Principal Component (Factor) 1

for the open space and agricultural land use types as well as the overlap of commercial and residential land use groups (**Fig. 3b**), indicated that PCDD/PCDF congener patterns were similar within each of these groups. This is in agreement with the statistical results obtained with soil TEQ concentrations where the different Off-RMA land use categories were separated into the same two groups. Furthermore, when the PCA data are plotted based on land use with spatial identifiers indicated, no clustering of samples relative to sampling region (NE, SE, NW, SW) in the Denver Front Range was observed (data not shown). Based on these results, the Off-site RMA land use categories were combined and reclassified from five to three designations:

- **Rural** land-use = combined **Agricultural** and **Open Space** land uses
- **Urban** land-use = combined **Residential** and **Commercial** land-uses
- **Industrial** land-use = remained as originally classified

Re-plotting the PCA results of the Off-RMA samples using the new sample identifiers produced little change in the occurrence of samples that fell outside their respective land use ellipses (see Fig. 3b). The spatial distribution of the outliers from the three consolidated land-uses was also examined to determine whether the outliers represented a significant area-specific cluster within any specific land-use. The clustering of such outliers in a single geographic location would indicate the possible presence of a point source of PCDD/Fs at that location. For the 'Industrial' and 'Urban' land-uses the outliers were fairly evenly scattered over the entire geographic range of the collected samples. A similar distribution of outliers was observed for the 'Rural' samples except for the presence of a small group of outliers in the eastern region study area. It can be concluded from this overall evidence that the occurrence of outliers in the Off-RMA sample groups is mostly a result of the relatively diffuse clustering of these samples and the lack of distinguishable profiles which define and discern the various sample groups. As such, it was not justifiable to remove any of these outliers (none were extreme) before performing subsequent analyses.

2.3 PCA of On-RMA samples

Mean concentrations of the 17 2,3,7,8-substituted PCDD and PCDF congeners in soils collected from within the RMA ranged from 416 to 1140 ng/kg dry weight (see Table 2). As was observed with the Off-RMA samples, the congener data were not normally distributed. As a result, all subsequent analyses were conducted with log transformed data. PCA analysis with the On-RMA data resulted in a total of three Factors that explained approximately 92% of the total variation. Factor 1 accounted for approximately 47% of the variability while Factors 2 and 3 accounted for 36% and 9% of the variability, respectively. An evaluation of the loading values indicated that PCDF congeners were more strongly segregated into Factor 1 with loading values all greater than 0.7. In contrast PCDD congeners were more strongly associated with Factor 2 with loading values greater than 0.7 with the exception of 2,3,7,8-TeD. 2,3,7,8-TeD was most strongly associated with Factor 3 ($r > 0.7$) while the contribution of the remaining PCDD and PCDF congeners was minor (< 0.5).

The On-site RMA soil congener data separated into three distinct elliptical centroids (**Fig. 4a**). The clustering was most pronounced for the WTP samples and less for the RMA site wide samples while the Historic Use samples seem to be

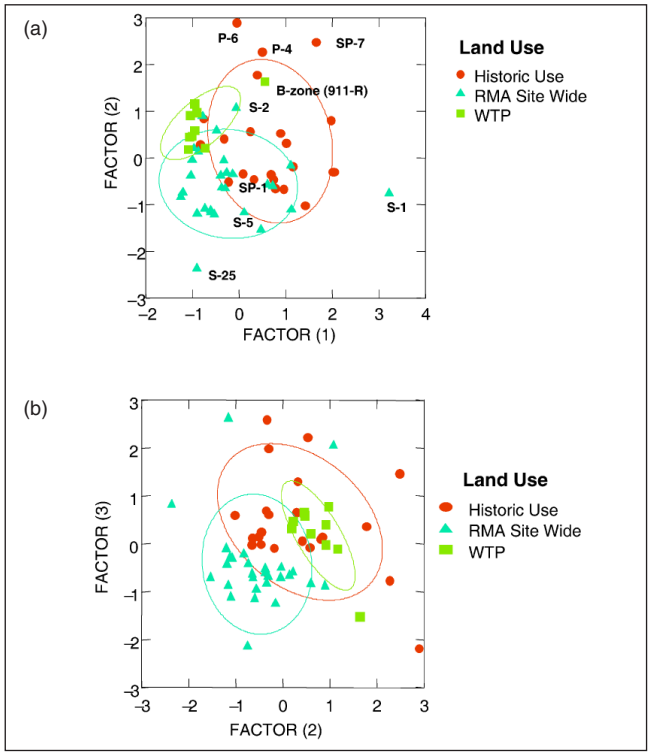

Fig. 4: Plot of principal component analysis (PCA) factors of PCDD/PCDF congeners in soils collected from On-RMA. Ellipses are sample based two standard deviation Gaussian confidence intervals. (a) PCA analysis was conducted with all soil samples. (b) PCA analysis conducted after deletion of 'outliers' from the dataset. Principal Component (Factor) 2 is plotted against Principal Component (Factor) 1

relatively dispersed. This clustering may be, to some extent, indicative of the sampling design and procedures used in the different studies. For instance, the WTP and Historic Use samples were collected as 5-point composites while the RMA Site Wide samples were collected from stratified random grab samples. In addition, the Historic Use samples were purposeful biased collections used to identify potential source areas of PCDD/PCDFs within the RMA. In viewing the elliptical centroids (see Fig. 4a) it is readily apparent that WTP and Site Wide samples overlap when comparing Factor 1 (PCDFs) to Factor 2 (PCDDs). The elliptical centroids for WTP and RMA Site Wide samples become more distinct when Factors 2 and Factor 3 (2,3,7,8-TeD) were contrasted (**Fig. 4b**) indicating that 2,3,7,8-TeD has some influence on the segregation of these samples based on congener profiles. Particularly noteworthy were the samples collected in the 'South Plants' (SP) and 'Purposeful sampling' (P) studies which comprise the Historic Use dataset (see Fig. 4a). These samples show considerably more variability than the WTP and RMA Site Wide samples. This variability results in a greater apparent number of outliers (seven samples) in the Historical Use samples. However, on closer examination, this result appears to be related to the relatively diffuse nature of the Historic Use cluster. Essentially, this greater number of outliers may simply be indicative of the fact that there was greater variability in the Historic Use cluster than in the other studies. This may be due to the fact that the purposeful samples consisted of biased 5-point composite samples that were collected from a variety of historic point sources. They would therefore be expected to demonstrate increased variability due to the potential for collection from distinct point source areas.

Four samples in the RMA Site Wide sample set were identified as outliers, S1, S2, S5, and S25 (see Fig. 4a). All of these samples were collected from 'core' or 'contaminated' locations (the six central sections of the RMA; sections 1, 2, 25, 26, 35 and 36 in Fig. 1). Only S5 was from an open space area on the eastern boundary of the RMA. Of these samples, S1 and S2 come from relatively central areas of RMA within the bounds of South Plants sample collection. S25 is from the northern central region of the arsenal close to samples P4 and P5, however these three samples do not form a 'cluster' in the analysis. For the Historic Use group, seven samples were identified as outliers (i.e. P3, P4, P6, P7, SP1, SP3 and SP7). However, while several of the data points for the South Plants area were outside the confidence ellipse, these samples (P3, P7, and SP3) were not removed for the subsequent analysis. In general the samples in this Historic Use group show a more diffuse distribution which would be expected for samples collected from an area where several minor potential PCDD/PCDF might have existed. For the WTP group, only the B-zone sample was identified as an outlier. Again, this result concurs with the soil TEQ data in that the average TEQ concentration in B-Zone was 7 ng TEQ/kg dry weight whereas the average TEQ concentrations in the other WTP zones ranged from 1 to 2 ng TEQ/kg dry weight. However, the source of this added contamination has not yet been identified (USEPA 2001c).

2.4 Comparison of On- and Off-RMA samples

The initial analysis of the On-RMA and Off-RMA samples indicated that there were significant differences in the relative concentrations of PCDDs and PCDFs between the sample groups. As a result it was determined that the use of one data-set as a training set for the other would not be a viable approach to compare the two sample groups. While this approach can be used to compare environmental samples to complex mixtures, it is necessary that the environmental samples contain some combination of the mixtures used in the training set. As a result, On-RMA to Off-RMA data sets were combined and a new PCA was performed. The initial PCA analysis returned two Factors that accounted for 84.5% of the variation in the data set. When the algorithm was forced to retain four Factors, 90.8% of all the variation in the dataset was explained. However, the fourth factor only explained an additional 2.7% of the total variation. In addition, the maximum loading for any congener in Factor 4 was 0.350 indicating that this Factor had little power to segregate samples. As a result, a final PCA was conducted that retained three factors accounted for approximately 88.3% variability in the data set. An evaluation of the congener loadings to each of the three Factors was similar to that observed for the On-Site RMA samples. For Factor 1, only PCDFs had loading values > 0.7 while PCDD had loading values that ranged from 0.22 to 0.67. In contrast, except for 2,3,7,8-TeD the congeners that had the greatest loading values relative to Factor 2 were PCDDs. This result indicated that there were significant differences in the PCDD/F congener profiles between the on-post and off-post sites. The greatest loading for Factor 3 was on 2,3,7,8-TeD with only minor loadings (r <0.300) for the other congeners.

Samples that were collected from the On-RMA locations were generally separated from the Off-RMA samples along Factor 1, whereas the Off-RMA samples were generally arranged along Factor 2 (**Fig. 5**). Since the more chlorinated PCDF congeners segregated into Factor 1 while Factor 2 is representative of the more chlorinated PCDDs it can be concluded that the 'signature' profile for the On-RMA samples is one that is dominated by PCDF congeners although all the clusters are not distinct. The presence of the relatively

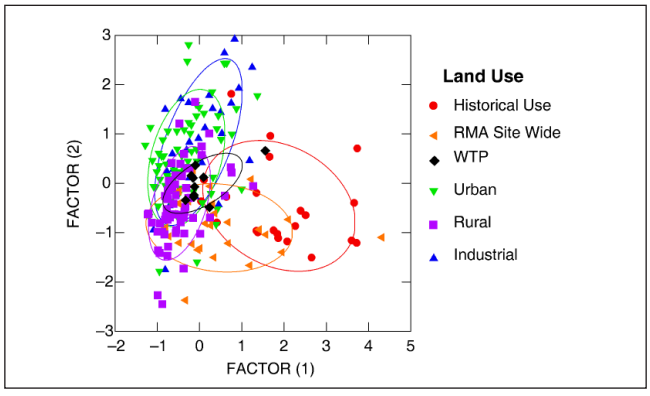

Fig. 5: Plot of principal component analysis (PCA) Factors of the patterns of relative concentrations of PCDD/PCDF congeners in soils collected from all On-RMA and Off-RMA land types. Principal Component (Factor) 2 is plotted against Principal Component (Factor) 1

greater PCDF signature at RMA is also apparent in some of the RMA Site Wide and Historic Use samples, as well as one of the WTP samples (B-zone). While the majority of samples from these groups clustered with the Off-RMA samples, the few outliers that were present are generally separated along the Factor 1 axis. This indicates that the presence of the RMA PCDF signature in these samples distinguishes them from the corresponding Off-RMA samples. In particular, this is the case for the RMA Site Wide samples that extend most completely into the RMA Historic Use centroid (see Fig. 5). In contrast, while the WTP samples were more closely associated with the Off-RMA samples, one outlier in this group (B-zone) was within the centroid for the Historic Use samples. This sample was collected near one of the public access points to the RMA and so is likely to have received treatment with chlorinated herbicides and pesticides that commonly contained PCDD/PCDF congeners.

A comparison of the Off-RMA 'Industrial' samples to On-RMA 'Historic Use' samples show two distinct profiles (**Fig. 6a**). The main axes for the sample centroids are close to orthogonal (at right angles), as would be expected for PCA analysis of data with differing congener profiles. The RMA samples were ordinated against Factor 1 that is represented by the more chlorinated PCDFs, while the Off-RMA 'Industrial' samples were ordinated against Factor 2 that is represented by the more chlorinated PCDDs. When On-RMA WTP and RMA Site Wide samples were compared to the modified Off-RMA 'Rural' samples, the majority of the WTP samples clustered more closely to the 'Rural' Off-RMA soil samples except for one outlier sample was more similar to the profile for the RMA Site Wide samples (**Fig. 6b**). The

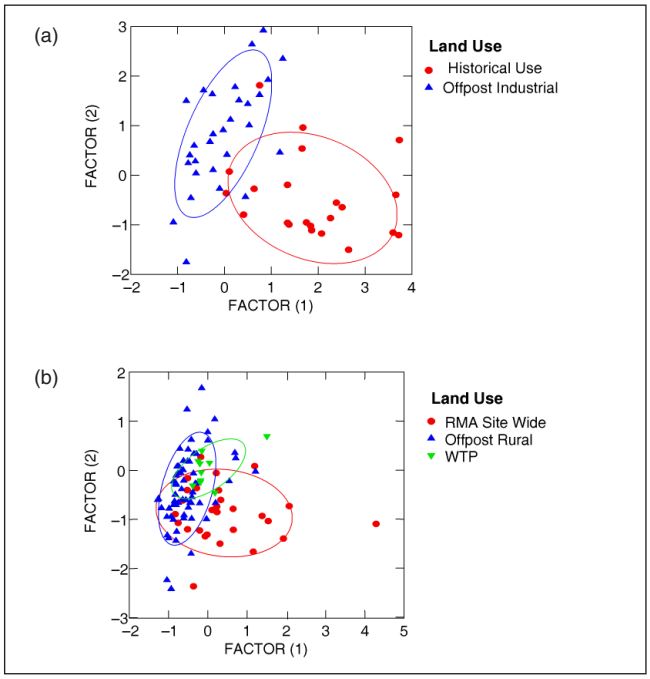

Fig. 6: Plot of principal component analysis (PCA) Factors of PCDD/PCDF congener patterns in soils from On-RMA and Off-RMA. (a) Comparison of Off-RMA 'Industrial' soils to On-RMA 'Historical Use' soils, (b) Comparison of On-RMA 'WTP' and 'Site Wide' soils to Off-RMA 'Rural' soils samples. Principal Component (Factor) 2 is plotted against Principal Component (Factor) 1

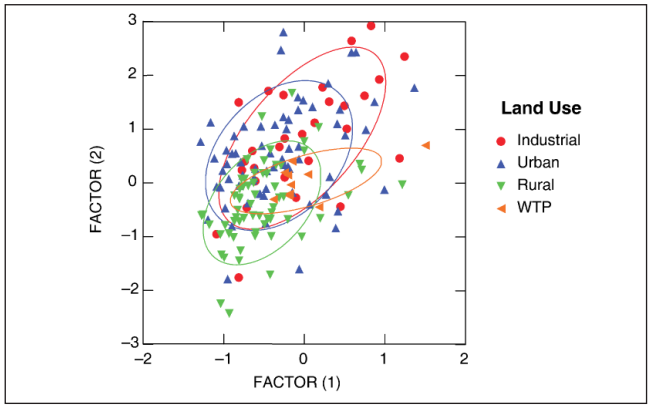

Fig. 7: Plot of principal component analysis (PCA) Factors of PCDD/PCDF congener patterns On-site 'WTP' soils to the modified Off-RMA sample land types. Principal Component (Factor) 2 is plotted against Principal Component (Factor) 1

RMA Site Wide samples that were separated from the Off-site 'Rural' cluster were separated by Factor 1 that was indicative of the presence of the On-Site RMA PCDF signature in these samples. Many of these samples were collected from either the 'Core' or on the boundary of the RMA core area. The core being that area of the RMA most influenced by historic uses of the site. These samples include S1, S2, S25, S26, S35, S36 that are located in the core area and S6 and S31 which border the South Plants area. It is therefore not surprising that these samples should be similar to those collected in the purposeful sampling of the more industrialized areas of the RMA. The majority of the WTP samples clustered with the 'Rural' Off-RMA samples except for the one outlier (B-zone) that was more similar to the RMA Site Wide samples (**Fig. 7**). When the WTP samples were compared to the modified Off-RMA land uses, it was apparent that all the points except the B-zone outlier lie within the centroids for these land-uses. This result indicates that the WTP samples are essentially indistinguishable from the 'Rural' land-use types as well as from the current Residential/Commercial lands in the Denver area. The exception to this general observation was seen for a small group of six samples collected from the combined 'Urban' human activity land-use group. These samples were separated from the other samples of the Urban group along Factor 3. Three of these samples were from commercial sites in the NW sector, one was from a residential area in the NW sector, one was from a commercial site in the SW sector, and the last sample was collected from a commercial site in the SE quadrant of the study area (see Fig. 1). The separation of these samples by Factor 3 suggests some influence from the tetra-chlorinated congeners that was not present in other samples from this land use type.

3 Concluding Remarks

In soils collected from On-site of the RMA, PCA identified a signature PCDD/PCDF profile that was dominated by the more highly chlorinated PCDF congeners. In contrast, samples from Off-RMA reference areas had congener profiles that were characterized by the presence of more highly chlorinated PCDD congeners which would be expected for samples influenced by a range of general human activities such

as traffic and light industrial applications. However, the presence of a PCDF signature for many of the RMA soil samples does not necessarily indicate a major source of these contaminants On-site. Rather, the relatively diffuse nature of the sample clusters would argue strongly against the presence of a single dominant source. Instead, the predominance of the PCDF congeners On-RMA is most likely indicative of the mixed industrial activities that historically have taken place on and near the site.

The conclusions drawn from this analysis are also supported other lines of evidence that indicate a relatively small source of PCDD/PCDF congeners located within the core area of RMA. The relatively small concentrations of PCDD/PCDFs in soil and biota samples make it difficult to categorically identify this source without additional analysis (Coady et al. 2001). By conducting the PCA described here it was possible to identify the congeners most indicative of the source and use this information to confirm the localization of the source. Based on the relatively small magnitude of the source it is anticipated that current remediation activities will result in removal of the source. This analysis also indicated that many of the 'open area' (peripheral site-wide) samples collected on the RMA are indistinguishable from the Off-RMA reference congener profiles. This finding was also true for samples collected from the WTP that were essentially indistinguishable from Off-RMA reference samples. In addition, total TEQ concentrations in soils collected from WTP were similar to those measured in soils collected from the Denver Front Range areas indicating that lack of a major source of PCDD/PCDF within the WTP zones of the RMA. In total, this evidence supports the contention that the WTP areas could be 'de-listed' from the other RMA areas and could be transferred to other authorities.

Acknowledgements. Statistical analysis was carried out under contract to US-EPA. Invaluable comments on earlier versions of the manuscript were provided by Dr Laura Williams, EPA Region 8, Denver CO and Dr Gerry Henningsen, H&H Consulting, Littleton, CO.

References

Banton MI, Klingensmith JS, Barchers DE, Clifford PA, Ludwig DF, Macrander A, Sielken Jr RL, Valdez-Flores C (1996): An approach for estimating ecological risks from organochlorine pesticides to terrestrial organisms at Rocky Mountain Arsenal. Hum Ecol Risk Assess 2, 499–526

Cacela D, Beltman DJ, Lipton J (2002): Polychlorinated biphenyl source attribution in Green Bay, Wisconsin, USA using multivariate similarity among congener profiles in sediment. Environ Toxicol Chem 21, 1591–1599

Coady KK, Jones PD, Giesy JP (2001): 2,3,7,8-Tetrachlorodibenzo-p-dioxin equivalents in tissue samples from three species in the Denver, Colorado, USA, metropolitan area. Environ Toxicol Chem 20, 2433–2442

De Wit C, Jansson B, Bergek S, Hjelt M, Rappe C, Olsson M, Andersson O (1992): Polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran levels and patterns in fish and fish-eating wildlife in the Baltic Sea. Chemosphere 25, 185–188

De Rosa CT, Brown D, Dhara R, Garrett W, Hansen H, Jones D, Jordan-Izaguirre D, O'Conner R, Pohl H, Xintaras C (1997): Dioxin and dioxin-like compounds in soil. Part 1: ATSDR Interim Policy Guideline. Toxicol Ind Health 13, 759–768

Dunn WJ, Stalling DL, Schwartz DL, Hogan JW, Petty JD, Johansson E, Wold S (1984): Pattern recognition for classification and determination of polychlorinated biphenyls (PCBs) in environmental samples. Anal Chem 56, 1308–1313

Filliben JJ (1975): The probability plot correlation coefficient test for normality. Technometrics 17, 111–117

Gnanadesikan R, Kettenring JR (1972): Robust estimates, residuals, and outlier detection with multiresponse data. Biometrics 28, 81–124

Ikonomou G, Fernandez MP, Knapp W, Sather PA (2002): PCBs in dungness crab reflect distinct source fingerprints among harbor/industrial sites in British Columbia. Environ Sci Technol 36, 2545–2551

Jackson JE (1991): A User's Guide to Principal Components. Wiley, NY, 569 pp

Looney SW, Gulledge Jr TR (1985): Use of the correlation coefficient with normal probability plots. The American Statistician 39, 75–79

Manninen H, Perki A, Vartiainen T, Ruuskanen J (1996): Formation of PCDD/PCDF: Effect of fuel and fly ash composition on the formation of PCDD/PCDF in the co-combustion of refuse-derived and packaging-derived fuels – Multivariate analysis. ESPR – Environ Sci & Pollut Res 3, 129–134

Meglen RR (1992): Examining large databases: a chemometric approach using principal component analysis. Mar Chem 39, 217–237

Peek DC, Butcher K, Shields WJ, Yost LJ, Maloy JA (2002): Discrimination of aerial deposition sources of PCDD and PCDF down wind from a pulp mill near Ketchikan, Alaska. Environ Sci Technol 36, 1671–1675

Rappe C (1994): Dioxin, patterns and source identification. J Anal Chem 348, 63–75

Rencher AC (1991): Methods of Multivariate Analysis. Wiley, NY, 627 pp

Schwartz TR, Stalling DL (1991): Chemometric comparison of polychlorinated biphenyl residues and toxicologically active polychlorinated biphenyl congeners in the eggs of Forter's tern (*Sterna fosteri*). Arch Environ Contam Toxicol 20, 183–199

Slymen DJ, de Peyster A (1994): Hypothesis testing with values below detection limit in environmental studies. Environ Sci Technol 28, 898–902

USEPA (1998): Approach for Addressing Dioxins in Soil at CERCLA and RCRA sites. OSWER Directive 9200.4-26. Memo from Timothy Fields Jr., April 13, 1998

USEPA (1999): Project plan for confirmation soil sampling at the Western Tier Parcel, RMA. Commerce City, CO., Prepared by USEPA Region 8 by ISSI Consulting Group, Inc., July 1999

US-EPA (2001a): Denver Front Range study of Dioxins in surface soil: Study 1: Characterization of Dioxin, Furans, and PCBs in Soil Samples Collected from the Denver Front Range. US-EPA Region 8, Denver Colorado

US-EPA (2001b): Denver Front Range study of Dioxins in surface soil: Study 2: Characterizations of Dioxin, Furans, and PCBs in Random Soil Samples Collected from the Rocky Mountain Arsenal. US-EPA Region 8, Denver Colorado

USEPA (2001c): Denver Front Range study of Dioxins in surface soil: Study 3: Western Tier Parcel. US-EPA Region 8, Denver Colorado

USEPA (2001d): Denver Front Range study of Dioxins in surface soil: Study 4: Characterization of Dioxins, Furans and PCB's in Soil Samples Collected from Historic Use Areas of the Rocky Mountain Arsenal. US-EPA Region 8, Denver Colorado

Van den Berg M, Birnbaum L, Brunstrom B, Cook P, Feely M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Petersen RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T (1998): Toxic equivalency factors (TEFs) for PCBs, PCDDs, and PCDFs for humans and wildlife. Environ Health Perspect 106, 775–792

Wenning RJ, Paustenbach DJ, Harris MA, Bedbury H (1993). Principal components analysis of potential sources of polychlorinated dibenzo-p-dioxin and dibenzofuran residues in surficial sediments from Newark Bay, New Jersey. Arch Environ Contam Toxicol 24, 271–289

Wenning RJ, Mathur DB, Paustenbach DJ, Stephenson MJ, Folwarkow S Luksemburg WJ (1999): Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans in Storm Water Outfalls Adjacent to Urban Areas and Petroleum Refineries in San Francisco Bay, California. Arch Environ Contam Toxicol 37 (3) 290–302

Wilkinson L, Blank G, Gruber C (1996): Desktop Data Analysis with SYSTAT. Prentice Hall, NJ, 798 pp

Yunker MB, Crentney WJ, Ikonomou I (2002): Assessment of chlorinated dibenzo-p-dioxin and dibenzofuran trends in sediment and crab heptopancreas from pulp mill and harbor sites using multivariate and index based approaches. Environ Sci Technol 36, 1869–1978

Received: January 14th, 2005
Accepted: June 28th, 2005
OnlineFirst: June 29th, 2005