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Title: 2022 Results for Avian Monitoring of Inorganic Elements and Organic Chemical Concentrations in Passerine Eggs and Nestlings Collected from Technical Area 16 Burn Grounds, Technical Area 36 Minie, and Technical Area 39 Point 6 at Los Alamos National Laboratory

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**2022 Results for Avian Monitoring of
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Burn Grounds, Technical Area 36 Minie, and
Technical Area 39 Point 6 at Los Alamos
National Laboratory**

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1 Summary

In 2022, nonviable avian eggs and one nestling were opportunistically collected at Los Alamos National Laboratory (LANL) near open detonation sites located at Technical Area (TA) 16 burn grounds, TA-36 Minie, and TA-39 Point 6. Samples were evaluated for inorganic elements (mostly metals) or per- and polyfluoroalkyl substances (PFAS).

- Three western bluebird (*Sialia mexicana*) egg samples were collected from TA-16 burn grounds.
- One deceased mountain bluebird (*Sialia currucoides*) nestling sample was collected from TA-36 Minie.
- One ash-throated flycatcher (*Myiarchus cinerascens*) egg sample was collected from TA-39 Point 6.

Concentrations of inorganic elements (i.e., mostly metals) observed in this study were compared with the regional statistical reference level (RSRL), which is the upper-level bounds of background concentrations (mean + three standard deviations = 99% confidence interval). Several inorganic elements were not detected in avian eggs. All of the inorganic elements that were detected were below the RSRL and the lowest observable adverse effect level (LOAEL), when available. No PFAS compounds were detected in the mountain bluebird nestling sample collected from TA-36, and the majority of PFAS compounds were not detected in the western bluebird egg sample from TA-16. These data suggest that inorganic element and PFAS concentrations in eggs and nestlings are not of ecological concern. More data are needed to make a robust assessment and to evaluate trends over time.

2 Introduction

In support of the Resource Conservation and Recovery Act permit process, LANL began annual avian monitoring in 2013 around TA-16 burn grounds and at two firing sites: TA-36 Minie and TA-39 Point 6. Biomonitoring is an important tool for assessing environmental contamination by analyzing chemicals or their metabolites from biological tissues (Becker 2003). Avian eggs and nestlings are useful as bioindicators because different species occupy many trophic levels. Additionally, the collection of nonviable eggs and/or nestlings that die of natural causes is non-invasive and is non-destructive to populations. Inorganic elements (i.e., mostly metals) and organic chemicals can pose risks of adverse effects to birds if exposed at high enough concentrations (Jones and de Voogt 1999). Birds can be exposed to chemicals through multiple routes, including diet, ingestion of soil, drinking water, and inhalation. Levels of some constituents in biological tissues can also indicate whether adverse effects could be expected (Gochfeld and Burger 1998). Examining population parameters along with tissue concentrations provides a more comprehensive and robust assessment of potential impacts caused by environmental pollution.

Polychlorinated biphenyls (PCBs) are manufactured chemicals that were used in industrial products; commercial production of these chemicals was banned in the late 1970s. Dioxins and furans are not manufactured—they are created as a result of the manufacturing of products (e.g., herbicides) or from the combustion of materials (e.g., coal, woods). Several congeners of PCBs, dioxins, and furans elicit similar toxic effects (i.e., immunotoxicity, carcinogenicity, and endocrine disruption) across several taxa, such as those caused by tetrachlorodibenzodioxin-2,3,7,8 (TCDD), which is the most potent in this class of

chemicals (Van den Berg et al. 2006). These congeners, like TCDD, have a high binding affinity to the aryl hydrocarbon receptor (Van den Berg et al. 2006). Several effects have been observed in birds when exposed to PCBs, dioxins, and furans, including effects on reproduction and development (Harris and Elliot 2011). The World Health Organization developed toxic equivalency factors for TCDD-like compounds that can be used to determine the relative potency—or toxic equivalents—of dioxin-like compounds for different classes of animals (i.e., fish, birds, and mammals) as well as to facilitate risk assessment for TCDD-like exposure (Van den Berg et al. 1998).

PFAS are a class of manufactured compounds that are used in many consumer and industrial products, such as cookware, food packaging, stain repellants, paints, and fire-fighting foams. PFAS compounds have useful properties, including repelling oil, stains, grease, and water, which contribute to their widespread use. Several thousand known PFAS compounds exist—some of which have been more widely used and studied than others—and these compounds have been manufactured since the 1940s. PFAS compounds are detected in the environment around the globe and have even been detected in avian tissues in remote areas, such as oceanic environments and from the Arctic region, where global deposition is the primary source of PFAS in the environment (Kannan et al. 2002; Martin et al. 2004). Toxicity data for PFAS compounds in avian ecological receptors are sparse.

Sources of inorganic elements include both anthropogenic and natural sources. Adverse effects, such as those on reproduction, in birds have been observed due to mercury and selenium exposures (Ohlendorf and Heinz 2011; Shore et al. 2011).

Inorganic elements, dioxins, and furans are of interest at open-detonation firing sites (TA-36 and TA-39) and at the burn grounds at TA-16 (Fresquez 2011). PFAS compounds are being monitored to contribute to site-wide characterization at LANL.

3 Objectives

The objective of this ongoing study is to document chemical concentrations in eggs and nestlings collected near TA-16 burn grounds, TA-36 Minie, and TA-39 Point 6 and to compare concentrations of inorganic elements, PCBs, dioxins, furans, and PFAS compounds observed in this study with the upper-level bounds of background concentrations, when available.

4 Methods

4.1 Sample Collection

Eggs and nestlings were collected from nest boxes when they were determined to be nonviable based on documented timing of known incubation periods for the species. In 2022, we collected a total of four nonviable egg samples and a deceased nestling at LANL near the TA-16 burn grounds (Figure 1), near open detonation site TA-36 Minie (Figure 2), and near TA-39 Point 6 (Figure 3). At TA-16 burn grounds, four nonviable western bluebird eggs were collected from one nest and was submitted as one composite sample, and two nonviable western bluebird egg samples collected from two separate nests were submitted as individual samples. At TA-36 Minie, one deceased mountain bluebird nestling was collected and submitted as an individual sample. At TA-39 Point 6, one ash-throated flycatcher egg sample was collected and submitted as an individual sample. All samples were collected June through July of 2022. Concentrations of chemicals in eggs and nestlings have been monitored annually at these locations since 2014.

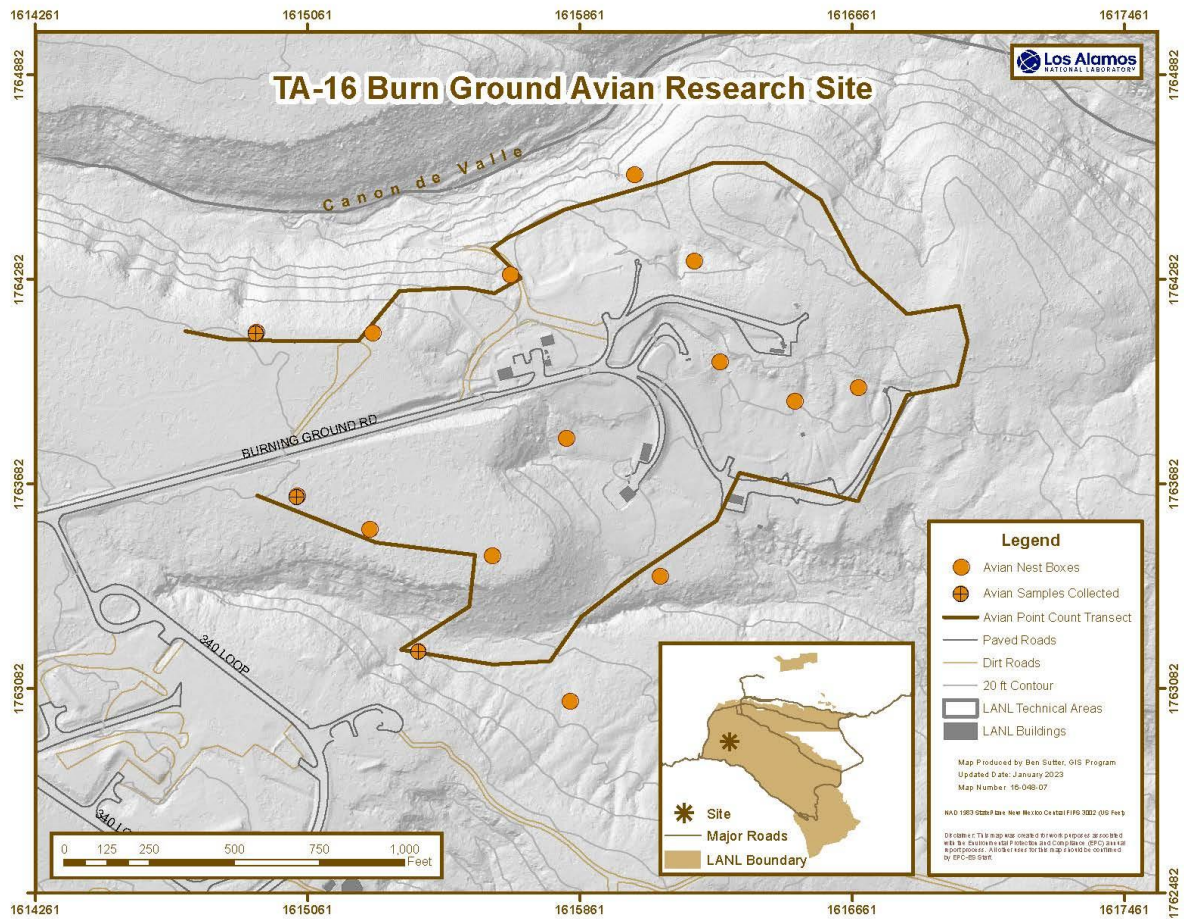


Figure 1. Avian nest box locations around TA-16 burn grounds.

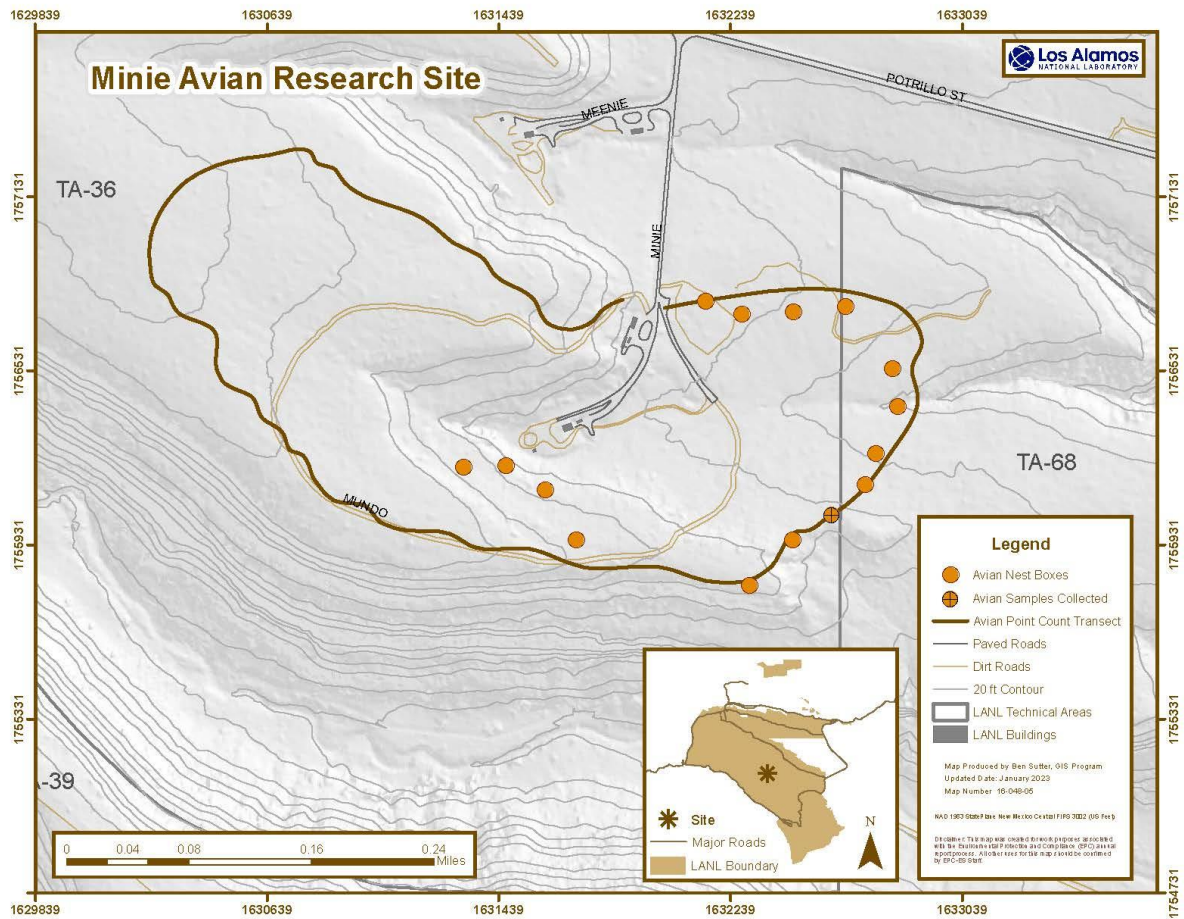


Figure 2. Avian nest box locations around TA-36 Minie.

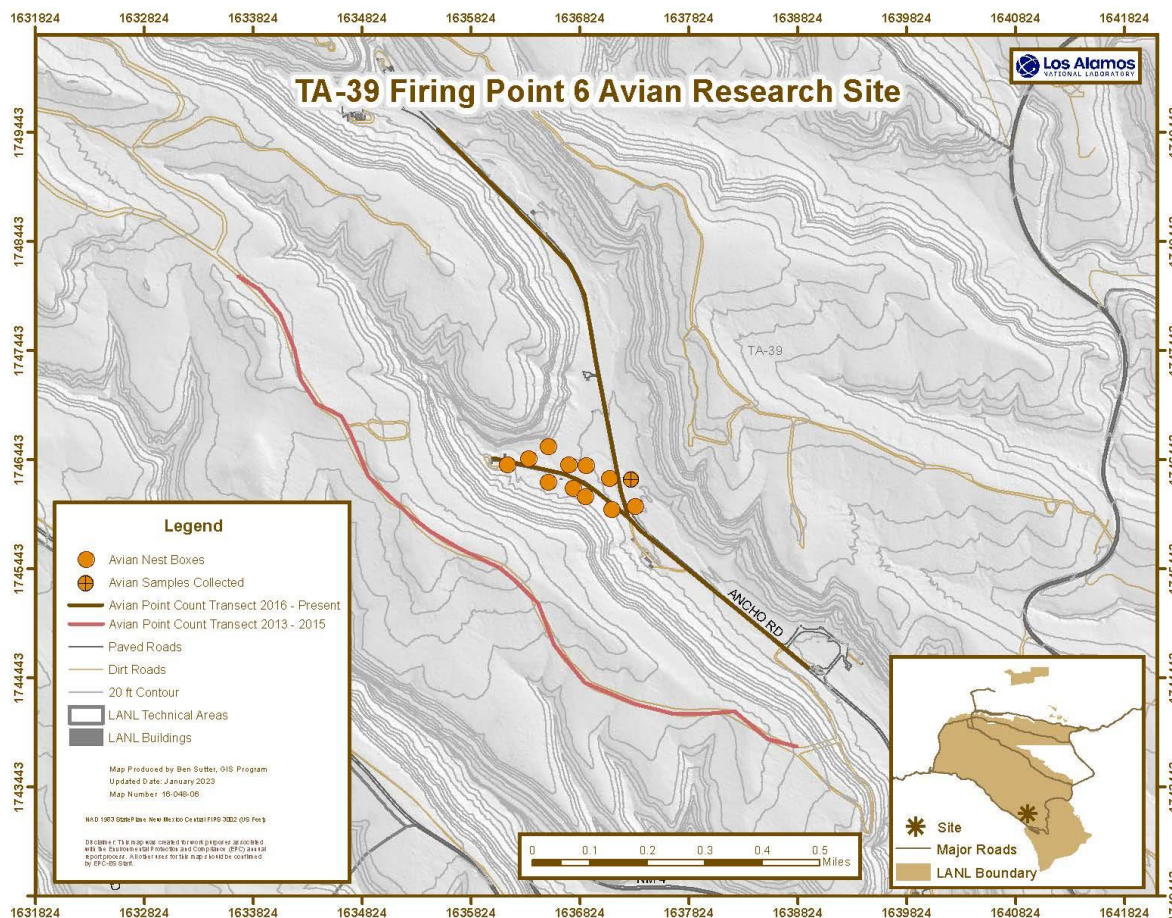


Figure 3. Avian nest box locations around TA-39 Firing Point 6.

4.2 Chemical Analyses

Due to limited sample mass, nonviable eggs were analyzed for total analyte list (inorganic elements) or PFAS only and were analyzed at GEL Laboratories in Charleston, South Carolina.

- Antimony, arsenic, cadmium, lead, selenium, silver, and thallium concentrations were measured in egg samples by inductively coupled plasma mass spectrometry (Environmental Protection Agency [EPA] SW-846 Method 6020).
- Aluminum, barium, beryllium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, sodium, vanadium, and zinc were measured by inductively coupled plasma atomic emission spectrometry (EPA SW-846 Method 6010B).
- Mercury was measured by cold-vapor atomic absorption procedure (EPA SW-846 Method 7471A).
- PFAS compounds were analyzed by liquid chromatograph triple quadrupole mass spectrometry (EPA:537M).

All inorganic element results were reported on an mg/kg (milligram per kilogram) wet weight basis, and PFAS compounds were reported on an ng/g (nanogram per gram) wet weight basis. No dioxin or furan congeners were analyzed due to limited sample masses.

4.3 Data Analyses

The 2022 results were compared with the RSRL, which represents natural and fallout levels of chemicals and are the upper-level bounds of background concentrations (mean + three standard deviations = 99% confidence interval). Regional statistical reference levels were calculated from nonviable eggs of western bluebirds and ash-throated flycatchers collected from Bandelier National Monument in 2021 and 2022 (n = 20 samples). Nonviable egg results were also compared with the LOAEL from peer-reviewed literature, when available.

Detectable concentrations of PFAS were compared with RSRLs, when available. The nestling RSRLs for PFAS were calculated from nonviable nestlings of western bluebirds at background locations from Bandelier National Monument in 2022 (n = 2). RSRLs for nonviable egg samples at background locations from Bandelier National Monument are not yet available due to limited sample size (n = 1).

5 Results and Discussion

Similar to previous years, many of the inorganic elements assessed in this study were not detected in passerine egg samples. Several elements are not maternally transferred (or very little is transferred) into eggs or do not accumulate in eggs, including cadmium (Leach et al. 1979; Stoewsand et al. 1986), lead (Pattee 1984), vanadium (White and Dieter 1978), and silver (Schwarzbach et al. 2006; Seiler and Skorupa 2001), which could explain why these elements were mostly not detected. Similarly, no PFAS were detected in the mountain bluebird nestling sample, and most PFAS assessed in this study were not detected in the western bluebird egg sample.

5.1 TA-16 Burn Grounds

The two separate nonviable western bluebird egg samples collected from nest boxes at TA-16 burn grounds did not contain detectable concentrations of aluminum, antimony, arsenic, beryllium, cadmium, cobalt, copper, lead, manganese, mercury, nickel, silver, thallium, or vanadium. All of the inorganic elements that were detected in the western bluebird egg samples were below the RSRLs (Table 1). Selenium concentrations were well below the LOAEL of 2.6 mg/kg (Ohlendorf and Heinz 2011). A mercury LOAEL is available of 1.9 mg/kg (Shore et al. 2011), but mercury was not detected in either of the western bluebird egg samples. No other LOAELs are available.

The one western bluebird composite egg sample (n = 4) collected from a nest box at TA-16 burn grounds was tested for 37 PFAS compounds; 4 compounds were detected at very low levels, including

- perfluoroundecanoic acid at 0.307 ng/g,
- perfluorononanoic acid at 0.317 ng/g,
- perfluorotetradecanoic acid at 0.733 ng/g, and
- perfluorotridecanoic acid at 1.02 ng/g.

An RSRL for PFAS in passerine eggs has not yet been calculated because only one egg sample was collected for PFAS from Bandelier National Monument. Although these four PFAS compounds are not as

well-studied as other PFAS compounds, such as perfluorooctanesulfonic acid (PFOS), a LOAEL for PFOS in avian eggs was determined at 92.4 ng/g (Dennis et al. 2021). All of the observed concentrations of PFAS compounds in the western bluebird at TA-16 were two orders of magnitude below the PFOS LOAEL. Additionally, the PFAS concentrations observed here are within the ranges observed in avian tissues from published studies, including studies that occurred away from point-source pollution and in the Arctic, where global deposition is the primary source of PFAS in the environment (Kannan et al. 2002; Martin et al. 2005). We are exploring other potential sources for some of the PFAS chemicals detected at LANL. Anticipated sources are atmospheric deposition and historical use of PFAS-containing materials.

Table 1. Detectable inorganic element concentrations (mg/kg wet weight) detected in two separate single egg samples collected near TA-16 burn grounds compared with RSRL. The RSRL is the upper limit background concentrations (mean + three standard deviations) for passerine eggs based on data in 2021 and 2022 (n = 20).

Element	Western bluebird (n = 1) SFB-22-255317	Western bluebird (n = 1) SFB-22-255318	RSRL
Barium	3.42	2.08	6.4
Calcium	1,820	569	9,382
Chromium	0.253	ND	1.18
Iron	43.1	ND	117
Magnesium	68.5	74	236
Potassium	1,680	1,800	4,145
Selenium	0.46	0.40	1.5
Sodium	2,190	1,940	4,029
Zinc	10.9	8.65	37.1

ND = Not Detected

5.2 TA-39 Point 6

The one ash-throated flycatcher egg sample collected from TA-39 Point 6 did not have detectable levels of several elements, including aluminum, antimony, arsenic, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, nickel, silver, thallium, or vanadium. All of the inorganic elements that were detected in the ash-throated flycatcher egg sample were below the RSRLs (Table 2 **Error! Reference source not found.**). Selenium concentrations were well below the LOAEL of 2.6 mg/kg (Ohlendorf and Heinz 2011). A mercury LOAEL is available of 1.9 mg/kg (Shore et al. 2011), but mercury was not detected in the ash-throated flycatcher egg sample. No other LOAELs are available.

Table 2. Inorganic element concentrations (mg/kg wet weight) detected in a single egg sample collected near the TA-39 Point 6 compared with RSRL. The RSRL is the upper limit background concentrations (mean + three standard deviations) for passerine eggs based on data in 2021 and 2022 (n = 20).

Element	Ash-throated flycatcher (n = 1) SFB-22-255320	RSRL
Barium	0.898	6.4
Calcium	643	9,382
Magnesium	64	236
Potassium	1,450	4,145
Selenium	0.48	1.5
Sodium	1,790	4,029
Zinc	5.8	37.1

5.3 TA-36 Minie

The one mountain bluebird nestling sample collected from TA-36 Minie did not contain any detectable levels of the 37 PFAS compounds that were analyzed. Similarly, no PFAS compounds were detected in nestling samples collected from Bandelier National Monument either.

6 Conclusions

The overall results indicate that the levels of constituents detected in eggs and nestlings are not likely to cause adverse effects in breeding bird populations from these study sites. Several constituents were not detected in the nonviable egg and nestling samples collected near TA-16 burn grounds, TA-36 Minie, and TA-39. All of the constituents that were detected were below RSRLs, and all were below the LOAELs, when available. These results suggest that the detectable concentrations observed here are not of ecological concern. More data from nonviable eggs and nestlings are needed to make a robust assessment and to examine trends over time. Evaluating avian nestling samples for high explosives is also of interest for future work as those data become available.

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8 Acronyms and Abbreviations

Acronym	Definition
EPA	Environmental Protection Agency
LANL	Los Alamos National Laboratory
LOAEL	lowest observable adverse effect level
mg/kg	milligrams per kilogram
ng/g	nanograms per gram
PCBs	polychlorinated biphenyls
PFAS	per- and polyfluoroalkyl substances
PFOS	perfluorooctanesulfonic acid
RSRL	regional statistical reference level
TA	Technical Area
TCDD	tetrachlorodibenzodioxin-2,3,7,8