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A Study of Economically Feasible Technologies to Remove Dioxin and Dioxin-Like Toxicants from Animal Co-Products

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PROJECT SUMMARY

Dioxin is a generic name often used to refer to 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD), one specific compound in the group of dioxins, but it is also used in reference to the chemically and structurally related polychlorinated dibenzo-para-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and the coplanar polychlorinated biphenyls (PCB). Within these classes there exists 75 PCDD, 135 PCDF, and 209 PCB congeners. The most toxic of these compounds is 2,3,7,8-tetrachlorodibenzo-p-dioxin, commonly referred to as TCDD. Chlorine substitution at the four corners of the base dioxin, furan and biphenyl structure are considered toxic or having 2,3,7,8-TCDD-like toxicity. When measuring the toxicity of other dioxins and dioxin-like compounds, TCDD is the congener to which toxic equivalents are normalized. There are 7 PCDDs, 10 PCDFs and 11 PCBs that fall in these categories of highest toxicity.

These organic compounds are considered to be present in virtually all natural environmental compartments either as a result of anthropogenic inputs from incineration of hazardous and municipal wastes, industrial chlorination processes, copper smelting, steel mill furnaces and wire reclamation incinerators, or from naturally occurring volcanic eruptions and forest fires. The presence of these toxic compounds in animal co-products is due to the ubiquitous distribution of the compounds and subsequent bio-accumulation in food webs. Humans can be affected by these toxic compounds through the consumption of food such as beef, pork, chicken, and fish. The consumption of eggs and milk can also lead to the exposure of dioxins to humans.

Numerous studies have focused attention on feed and food stuffs as major sources of dioxin and dioxin-like toxicants in the human food chain. These toxic compounds are present in most ingredients that are included in commercial feeds. Ingredients of animal and mineral origin typically may contain high concentrations of a variety of contaminants, and when animal co-products are reprocessed as animal feed, exposure to humans may increase. The most important exposure route to humans is likely fat-containing animal foods and some sea foods.

Animal parts are ground up as fat and bones are separated from each other to form many by-products used by humans. The use of these by-products may lead to adverse health effects if by-products are significantly contaminated with the toxic compounds.

Since 1995, several in-depth national and international studies have been conducted to measure the concentration and TEQs for dioxin and dioxin-like contaminants in a variety of food categories, including meat, poultry, milk, eggs, fats and oils, fish, and fruits and vegetables; and animal feedstuffs. Parts per trillion TEQs seldom exceed 2, a limit currently adopted in the European community for fats and oils. This limit may be reduced in 2006. Depending on limits promulgated in the United States, there may be the potential for some concern.

Only now are these compounds really being studied and the importance of their removal from the environment realized. Research is ongoing to find ways to degrade these compounds in a safe and economically efficient manner. Microorganisms are quite possibly the best solution. Several reports have proven the ability of the bacteria *Pseudomonas resinovorans* strain CA10 and *Staphylococcus auriculans* strain DBF63 to degrade dioxins and dioxin-like compounds because of their composition of angular dioxygenases.

Follow-on research to understand the mechanisms and kinetics of enzymatic catalysis could lead to plausible degradation technologies to significantly reduce the presence of dioxin-like contaminants in a variety of matrices including animal coproducts.

PROJECT OBJECTIVES

The primary objectives of this investigation were to (1) provide a complete summary of available data regarding the level of dioxin and dioxin-like toxicants in rendered animal co-products, (2) determine the feasibility of removing or reducing toxicants to acceptable levels in animal co-products that remain in the human food chain or those

destined for human use other than direct consumption (cosmetics, etc.), and (3) conduct cost-benefit analyses for each of the protocols deemed as feasible technologies to reduce or eliminate dioxin and dioxin-like toxicants from animal co-products.

SUMMARY OF PERTINENT LITERATURE

These organic compounds are considered to be present in virtually all natural environmental compartments either as a result of anthropogenic inputs from incineration of hazardous and municipal wastes, industrial chlorination processes, copper smelting, steel mill furnaces and wire reclamation incinerators, or from naturally occurring volcanic eruptions and forest fires. The ubiquitous and persistent nature of these hazardous organic compounds in environmental compartments is well documented in the recent research literature, and as a result they have been detected in many types of plant and animal tissue, (U.S.: Schecter, et al., 1997, 2001, Fiedler, et al., 1998; India: Kumar, et al., 2001; Spain: Lopez-Leiton, et al., 2001, Eljarrat, et al., 2002; Switzerland: Schmid, et al., 2002; France: Roche, et al., 2000, 2003; Vietnam: Schecter, et al., 2003; and Germany: Rimkus and Wolf, 1993).

The most important pathway for initial entry into the food chain is atmospheric deposition onto edible plants, deposition into water ways, and deposition onto soil and subsequent ingestion by animals. Numerous studies have focused attention onto feed and food stuffs as major input sources of dioxin and dioxin-like toxicants into the human food chain/web. Commercial feeds are typically prepared from ingredients of plant, animal and mineral origin. Animal origin ingredients typically include animal fat and protein, fish oil, fish meal, and meat and bone meal. Many of these ingredients are animal co-products. Additives of mineral origin, widely employed as binders and anticaking agents, include bentonite, kaolin, zeolite, sepiolite, and magnesite. Karl, et al. (2003) found a direct correlation between the dioxin concentration in feed and the resulting concentration in muscle tissue fat in farm raised trout. Vartianen and Hallikainen (1994) reported that high levels of dioxin in fish products, used in feed for poultry, accounted for egg and poultry contamination. Eljarrat, et al. (2002) noted high

levels of dioxin in feed samples of animal origin. Easton, et al. (2002) and Jacobs, et al. (2002) reported significant contamination of salmon aquaculture feed and fish oil components of feed. Other researchers have reported that additives used as binders and anticaking agents contain high concentrations of dioxin and dioxin-like toxicants, (Schmid, et al., 2002; Abad, et al., 2002; Ferrario, et al., 2000; Eljarrat, et al., 2002; Fiddler, et al., 2000).

Human exposure to these toxic agents can be from inhalation from the atmosphere (including particulate forms), or ingestion of plants and animals. Inhalation and drinking water have generally been ruled out as major exposure pathways for humans. The most important exposure route for humans is likely fat-containing animal products and some sea foods. Animals in feed lot operations, on high-roughage diets, or that ingest contaminated soil are the most likely to accumulate toxic residues. Aquaculture operations that feed animal by-products (including recycled fish by-products) and fish oil also result in the accumulation of toxic organic compounds in the fat tissues of farm raised fish.

Due to the hydrophobic nature of these toxicants, they tend to accumulate in the adipose tissue of animal species, including humans. Rendering animal processing wastes into fats, proteins, and solid materials does not remove the contaminants from the rendered co-products, but tends to accumulate them in the more hydrophobic matrices (fats). Technologies to remove toxicants from the rendered co-product categories would therefore be favorably considered in-lieu of an aggressive monitoring program to ensure product safety. Such technologies are at present unknown.

The term dioxin refers to hundreds of compounds which can be found in many places in the environment. These dioxins and dioxin-like compounds encompass polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polybrominated dibenzo-p-dioxins (PBDDs), polybrominated dibenzofurans (PBDFs), and polychlorinated biphenyl's (PCBs). As stated before, there are hundreds of these compounds present in the environment, such as 75 PCDD compounds, 135 PCDF

compounds, 75 PBDD compounds, 135 PBDF compounds, and PCBs having 209 known compounds. Dioxin-like compounds are so called because they have similar chemical structures, physical properties, and a common battery of toxic responses. Only seven of the 75 CDDs and BDDs have dioxin-like toxicity and only 13 of the 209 PCBs have dioxin-like toxicity. They are hydrophobic and lipophilic, giving rise to their accumulation in the fats of animals and humans. The most toxic dioxin compound, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is used as the reference point in which the toxicity of all other dioxin compounds' toxicity is measured. TCDD is assigned a value of one, the maximum toxicity, with all other compounds assigned a number relatively proportional to their toxicity in relation to TCDD. Dioxin concentrations are reported in TEQs, or toxic equivalents, which show the toxicity-weighted masses of mixtures of dioxins. Figure 1 shows the structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin and a dibenzofuran.

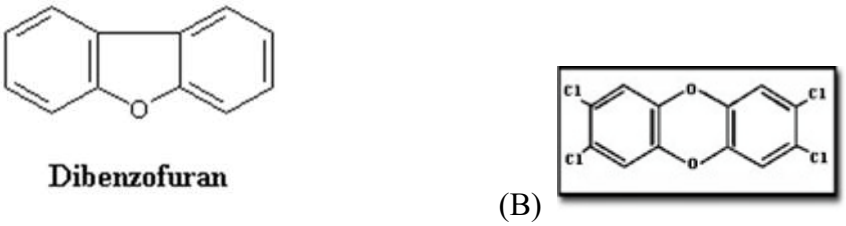

FIG 1. Structure of a typical dibenzofuran (A), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (B).

There are many sources for these highly toxic compounds. They can form from combustion sources such as waste incineration, burning of fuels, and forest and building fires, from metal smelting, refining and processing sources, as by-products from chemical manufacturing, from biological and photochemical processes, and from reservoir sources such as soils, sediments, biota, and water. The pictorial in Figure 2 dipicts and differentiates U.S. dioxin sources from 1987 to 2002/4. A major shift in exposure from incineration to backyard burning is noted.

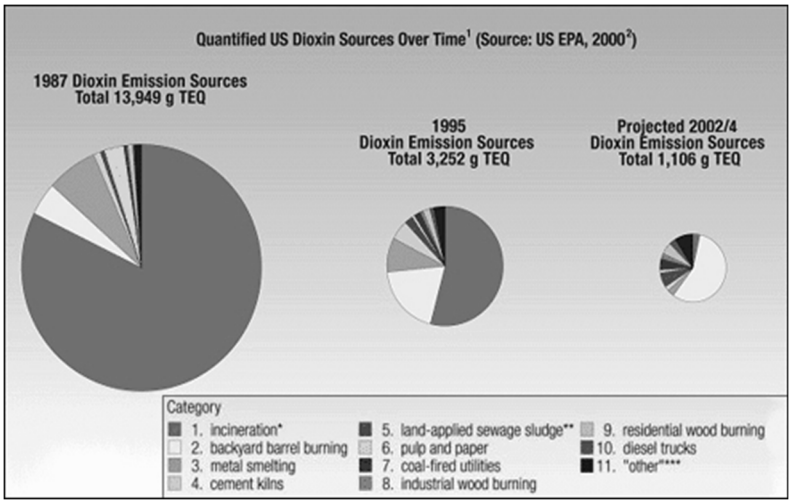

FIG. 2. US dioxin sources from 1987 – 2002/4. The leading source has shifted from incineration to backyard burning of trash.

Human exposure is almost solely from reservoir sources as they can redistribute and circulate the dioxins. Figure 3 shows total daily dioxin exposure as it relates to a typical American diet. Beef ingestion is shown as the leading source of dioxin exposure to humans on a daily basis

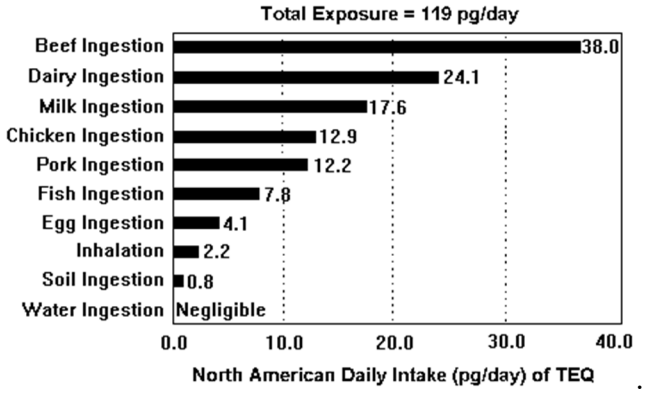

FIG. 3. Total daily dioxin exposure as a result of eating a typical North American diet.

The specific products in which this exposure can come from is shown in figure 4. Here it can be seen freshwater fish exhibit the highest level of TEQ in the U.S. food supply, although they are not the source for the highest exposure of dioxins to humans, only because beef is more common in a typical North American diet.

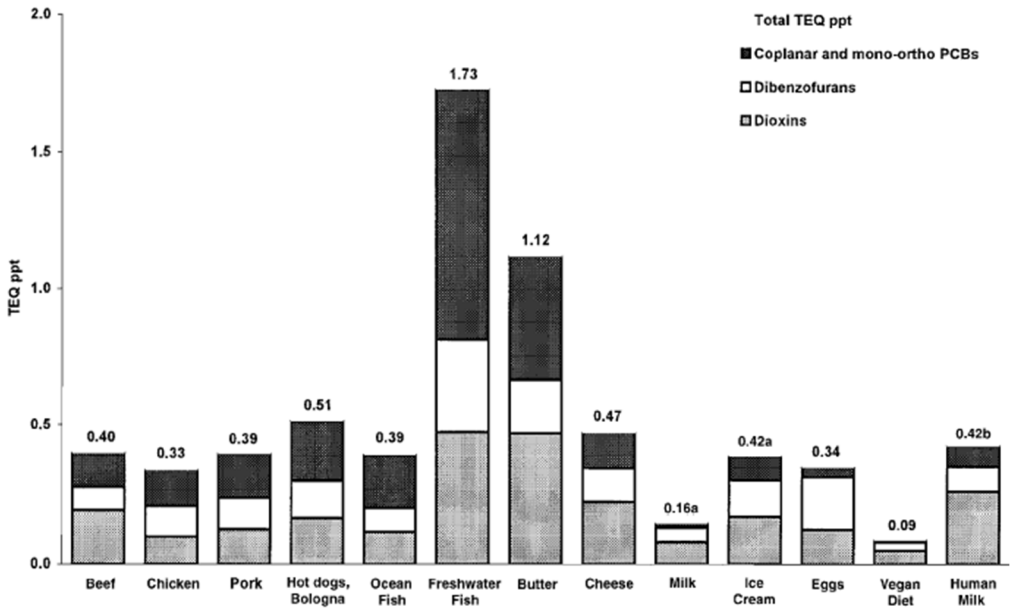

FIG. 4. Levels of Dioxin in U.S. Food Supply.

Dioxins and dioxin-like compounds have numerous adverse health effects. The U.S. National Toxicology Program and the International Agency for Research on Cancer have each linked dioxin exposure to cancer. Some of the other well documented health effects are birth defects, diabetes, learning disabilities, damage to the immune system and inability to maintain pregnancy. These effects can be seen when exposed to levels as low as 100 times less than the cancer-causing levels. It should be noted there is likely no safe dose of dioxin exposure, i.e., no threshold level. One major problem with dioxins is the difficulty humans possess in ridding them from the body. The only way males can get rid of dioxins is to let them break down by their half-life. Typical half lives are on the order of years and center around the bodies ability to convert the hydrophobic compounds to more water-soluble hydrophilic compounds to facilitate excretion from the body. The female body can eliminate the body burden of toxic compounds by crossing of the placenta into the growing infant or through their breast milk. Either route is extremely harmful to the developing infant or young child.

In order to understand one important means of transport of dioxins to humans, it is essential to know more about the process of animal rendering. Animal rendering is the transforming of waste from the meat industry to useable products. This process accounts for over 9 billion pounds of reclaimed animal fat per year. There are two types of plants associated with rendering. The first type, which operates in conjunction with animal slaughter houses or poultry processing plants, is known as an integrated rendering plant. Plants that get their raw materials from offsite are known as independent rendering plants. These plants obtain their by-product materials such as grease, blood, feathers, offal (bladder, diaphragm, udder, intestines, etc.), and whole animal carcasses from many different sources. These sources could be butcher shops, supermarkets, restaurants, slaughterhouses, farms, and feedlots to name a few. Each of the types of plants mentioned above can operate two different processes of animal rendering: edible and inedible. Edible rendering plants use fatty animal tissue to make edible fats and proteins. Since dioxins concentrate in the fats of animals, this can lead to human exposure. Inedible rendering plants process the rest of the animal to form the many by-products used by humans. Examples of these by-products include: numerous pharmaceutical products, margarine, soap, chewing gum, food preservatives, glue, brushes, rugs, and many other products used almost daily by humans. Recently, a major cause for concern recently has been the dioxin contamination of pet food and animal feed. Table 1 shows the rendered product volumes as a result of the animal rendering process.

Table 1. Rendered Product Volumes - 2000	
Ingredient	**Million, lbs**
Meat & bone meal	6,652.4*
Poultry meal	3,073.5*
Blood meal	226.5*
Feather meal	1,200.0*
Tallow/greases	7,096.1**
* Sparks, 2001	
** US Census Bureau, 2001	

Table 1. Rendered Product Volumes – 2000.

This use of the inedible parts of the rendered animals should be closely monitored as it is placing the dioxins back into the food chain, and allowing further bioaccumulation.

Most animal rendering plants use the same system to produce the by-products. Diagram 1 shows the continuous rendering system beginning as the raw material reaches the plant. The raw material is stored temporarily in bins, after which it is conveyed from these bins and across a magnet to remove ferrous metal contaminants. The raw material is reduced to uniform particle size by a grinder. This allows for better handling and improved heat transfer during the cooking step. The raw material continues to a cooker where it is heated to 250-280˚ F. This separates the fat from the protein and bone. Another conveyor separates the liquid fat from the solids and each continues to either storage containers or goes on to further processing.

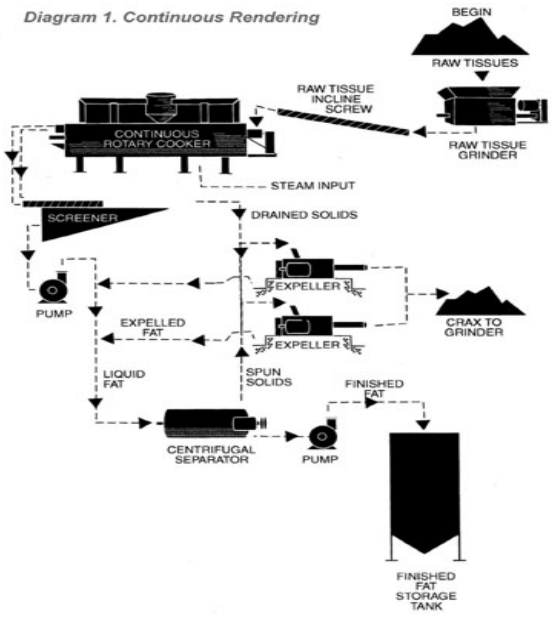

Diagram 1. The flow chart for the continuous rendering process. This is the most commonly used process among animal rendering plants.

Several national and international studies have been concluded in recent years summarizing concentrations and TEQs for dioxin and dioxin-like in a variety of food categories, including meat, poultry, milk, eggs, fats and oils, fish, and fruits and

vegetables; and dairy feedstuffs. Of these and pertinent to an evaluation of contaminant levels in the U.S. market are the investigations of Schecter and Li, (1997) measuring the concentration of D/Fs and dioxin-like PCBs in US fast foods; Schecter et al. (1997) measuring the concentration of the above contaminants in food samples collected at supermarkets; Kim, et al. (2004) comparing seven indicator PCBs and three coplanar PCBs in beef, pork, and chicken fat; Winters, et al. (1996) measuring dioxin-like compounds in beef; Huwe, et al. (2004) reporting PCDD/Fs and coplanar PCBs in the meat and poultry supply; Schuda, et al. (2004) evaluating dioxin in cow's milk; and Lober, et al. (2004) evaluating levels of dioxin-like compounds in dairy feeds.

Schecter and Li, (1997) and Schecter et al., (1997) measured dioxin plus dibenzofuran toxic equivalents for a variety of fast-food including burgers, pizza, chicken and ice cream. Typical TEQs ranged from 0.01 to 0.03 TEQ pg/g actual weight. Similarly, food samples were collected from supermarkets across the United States, pooled into 12 groups and analyzed for dioxins, furans and PCBs. Total PCDD/F/PCB TEQ values ranged from 0.07 pg/g for the vegan diet to 1.43 pg/g on a whole weight basis for the freshwater fish sample. These data were measured from samples collected from all major U.S. geographical regions.

Kim, et al. (2004) reported concentrations of indicator PCBs averaging 466, 384, and 460 pg/g fat for 53 beef, 66 pork, and 30 poultry samples respectively. TEQs/g fat were also reported for the coplanar PCBs (dioxin-like PCBs) for the beef, pork, and poultry samples and were 0.38, 0.20, and 0.25 pg TEQ/g fat respectively. Dioxin data was not included in this investigation.

Winters, et al. (1996) reported on the completion of one of the first statistically designed surveys of the occurrence and concentration of PCDD/F in the fat of beef raised for human consumption in the U.S. Back fat samples were collected from 63 carcasses. The mean I-TEQ, (lipid adjusted) concentrations were 0.35 and 0.89 pg/g for non-detects treated as 0 or assigned a value of ½ the detection limit respectively.

Huwe et al. (2004) conducted a follow-on survey to the USDA/USEPA survey completed in the mid 90's. Approximately 510 samples were collected from the back fat of heifers and steers, from the belly fat of gilts and barrows, and from the abdominal fat of chickens and turkeys. The importance of this data set relative to the overall objectives of this project is that 92% of the samples had TEQs < 1.0 pg/g (nd=DL/2), and 97% were < 2.0 pg/g.

Schuda et al. (2004) reported PCDD/F pg TEQ/g lipid for milk samples collected in January 2001 and a previously collected (July, 2000) milk sample data set (Schaum et al. 2003). Schuda normalized the Schaumn data set to the lipid content and reported along with the January 2001 data set. Milk samples were collected from 8 regional locations across the U.S. The regional mean TEQs were 0.68 and 0.64 pg/g lipid for the July, 2000 and the January, 2001 sampling respectively.

Lober et al. (2004) measured and reported PCDD/F and PCB ppt TEQs from dairy feed total mixed rations (TMR) collected from several different regions of the country. TMR TEQ, PCDD/F/PCB pg/g ranged from 0.02 from the Florida sample set to 0.09 from the Michigan sample set.

FEASIBILITY OF REMEDIATING CONTAMINANT LEVELS

At present an effective process to remove dioxin-like contaminants from animal coproducts (fats and oils) without degrading the product is unknown. However, a potentially plausible remediation technique centers on the ability of microorganisms to degrade the contaminants. If microorganisms are capable of degrading the contaminants, they must first synthesize the enzymes to catalyze the degradation. Since it is not practical to treat the fats and oils with microorganisms, it is plausible to isolate the cellular enzymes and subsequently add the enzymes to the fats and oils to achieve enzymatic catalysis. To remove the enzymes from the fats and oils before

further processing to edible or inedible products, system temperatures could be elevated to denature the then unwanted enzymes.

The main focus of this phase of the investigation is the degradation of dioxins and dioxin-like compounds by microorganisms. Again, this approach is not directly applicable to the remediation of the contaminants in fats and oils, an application of the microbial approach using enzymes to catalyze the transformation of these contaminants, may be plausible.

Two microorganisms in particular were chosen to concentrate on coming from several different published research studies. The bacteria Staphylococcus auriculans strain DBF63 and Pseudomonas resinovorans strain CA10 were chosen because of the similarities between the two. Both exhibit angular dioxygenases with different features which are used in the degradation of chlorinated dibenzofurans and dibenzo-p-dioxins. Several studies several studies have been conducted to determine degradation routes and mechanisms for chlorinated dibenzofurans (CDFs) and chlorinated dibenzo-p-dioxins (CDDs). They also have investigated the potential of strains DBF63 and CA10 to cooxidize mono-, di-, and triCDF/Ds. An in depth look at these studies is needed to understand the mechanisms by which each of these bacterial strains are able to degrade the resistant compounds.

Staphylococcus auriculans strain DBF63 was isolated from soil in the eastern part of Japan. Twenty strains from 592 soil samples were analyzed with strain DBF63 being chosen because of its ability to grow on dibenzofuran as the sole source of carbon and energy. It is also capable of cometabolizing dibenzo-p-dioxin. Cometabolism is the metabolic transformation of a substance while a second substance serves as primary energy or carbon source. The strain can grow on fluorine in the same manner, although growth on dibenzofuran was greater. The study by Monna, et al.(1993) identified dibenzo furan (DBF) degradation products by using thin-layer chromatography. Four major spots showed and these products were isolated by silica gel column chromatography and eluted with chloroform. After recrystallization, DBF-P2 was found

to be a major product and later identified as salicylate. Salicylate is a metabolic intermediate of DBF degradation and is shown to continue degradation by this strain and support its growth. This study proposed degradation pathways and also identified some of the chemical structures of the products of this degradation. It was shown that salicylic acid and gentisic acid accumulated by this strain growing on DBF. The group also introduced and inhibitor of metapyrocathase, pyrogallol, that accumulated 2,2',3-trihydroxybiphenyl. At the time of this research, it was not known whether gentisic acid was formed by hydroxylation of salicylic acid or from other metabolites. While this experiment shows the degradative pathway for DBF, it sets up the next experiment by looking for further degradation of dibenzo dioxin (DD).

In follow-up research of Staphylococcus ariculans strain DBF63, the angular dioxygenase of the bacteria was closely studied. The angular dioxygenase can act on the angular position adjacent to the oxygen atom of DBF and DD skeletons. This will also be looked at later when the bacteria Pseudomonas resinovorans strain CA10 is discussed. Kasuga et al. cloned, sequenced, and characterized genes encoding terminal oxygenase components (dbfA1A2) of DBF 4,4a-dioxygenase (DFDO). These genes catalyzed the oxidation of DD to 2,2',3-trihydroxydiphenyl ether as shown in Figure 5. This figure also shows how toxicity is reduced by the ring cleavage because angular dioxygenation produces unstable hemiacetal compounds that rearomatize spontaneously, prompting cleavage of the ether bond. Staphylococcus ariculans strain DBF63 was first thought to have no degradative ability for 2,7-dichlorodibenzo-p-dioxin (DCDD). However, further studies showed this compound to be degraded up to 10-25%, but not by DFDO attacking the substrate. A monohydroxylated derivative of 2,7-DCDD was detected leading to the assumption that it was degraded by other oxygenases or hydroxylases in strain DBF63.

Pseudomonas resinovorans strain CA10 was studied by Habe, et al. (2001) during their same study of Staphylococcus ariculans strain DBF63. It was selected because of its utilization of carbazole (CAR) as its sole carbon, nitrogen, and energy source. Genes encoding CAR 1,9a-dioxygenase (CARDO) were isolated and analysis showed that it

consists of a single proton of terminal oxydase (CarAa), ferredoxin (CarAc, and ferredoxin reductase (CarAd). CARDO uses its broad substrate range to convert DD and DBF to 2,2',3-THDE and 2,2',3-THB, respectively. Figure 6 shows this conversion. As seen in strain DBF63, CARDO was also shown to degrade 2,7-DCDD, producing a metabolite. A follow-up study showed CA10 cells decreased rapidly in soils with pH 6, but maintained a high cell density at pH 7.3 (Widada, et al. 2002). This proved CA10 is strongly influenced by pH and also organic matter. Not only can carbazole 1,9a-dioxygenase catalyze dibenzo-p-dioxin and dibenzofuran, it was shown to catalyze a variety of other aromatic compounds such as biphenyl, naphthalene, dibenzothiohene, and phenanthrene. The study mainly focused on CA10's ability to degrade 2,3-DCDD and to look at its survival in the soil microcosm. A visual marker form the jellyfish Aquoria Victoria, green fluorescent bacteria (gfp), was used to estimate survival and growth. This marker was chosen because the gfp in marine eukaryotes should not be present in soil microorganisms. It also proved to be very durable as it was stably maintained in CA10 for more than 40 generations on non-selective medium, nor did it have any apparent adverse effects on the growth of CAR, meta-cleavage capability for 2,3-dihydroxybiphenyl, or degrading ability for CAR. Two soil types were used in the study. Nose-granitic (NG) and nose-granitic upland (NGU) soils were taken from Nose-cho, Osaka Prefecture, Japan and exhibit different characteristics including, but not limited to pH, total carbon, total nitrogen, sand, silt, and clay. No significant CAR degradation was detected in NG soil after 21 days, but CAR was rapidly degraded in NGU soil. However, 2,3-DCDD was degraded by CA10 in NG soil and the extent was dependant on initial CA10 density. The most intriguing data from this experiment was obtained by inoculating 2,3-DCDD contaminated soil every 2 days with CA10 cells and observing a near 100% degradation within 14 days. Therefore, the potential for enhanced degradation of carbazole and 2,3-DCDD was clearly shown in this set of experiments. Further studies should be conducted, but this data is very promising for the future use of Pseudomonas resinovorans strain CA10 for bioremediation of carbazole and dibenzo-p-dioxin contaminated soils.

Studies of both bacterial strains DBF63 and CA10 were conclusive enough to predict possible sites of attack by angular dioxygenases as well as products which can be seen in figure 6. In summary, these angular dioxygenases with different features have an important chlorine substitution pattern which gives the strains DBF63 and CA10 the ability to cooxidize various mono-, di-, and tri-CDF/Ds.

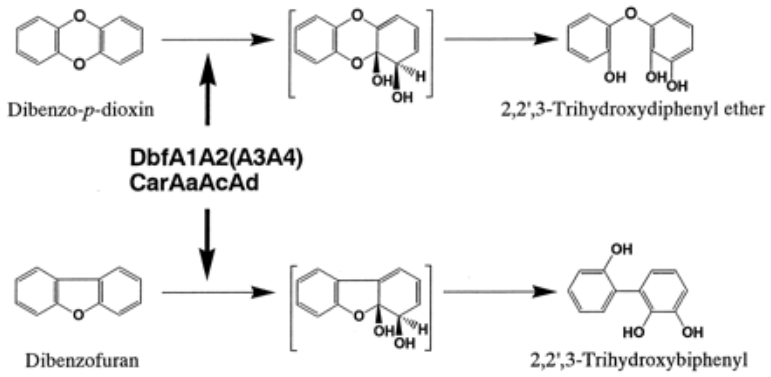

FIG. 5. Conversion of dibenzo-p-dioxin and dibenzofuran by dibenzofuran 4,4a-dioxygenase and by carbazole 1,9a-dioxygenase.

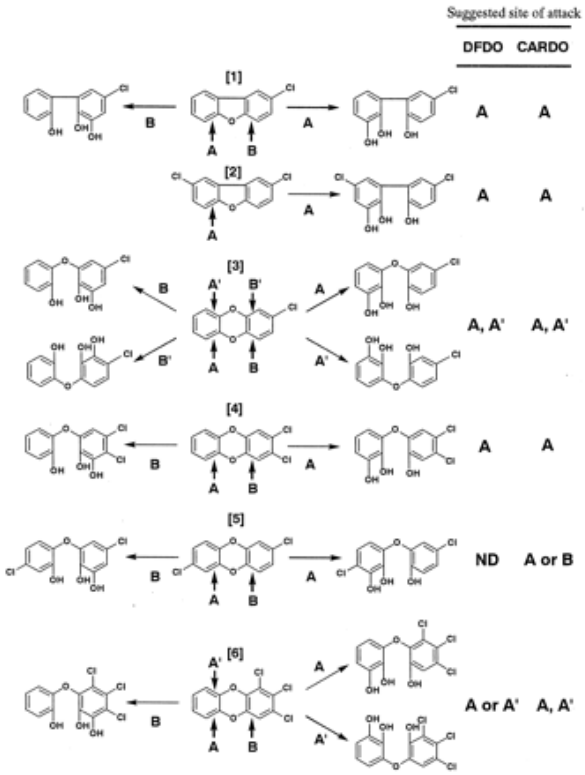

FIG. 6. The proposed initial attack on the tested CDFs and CDDs by DFDO and CARDO. A or A' show the suggested sites of attack by angular dioxygenase on the nonsubstituted rings and B or B' shows the suggested sites of attack on the halogen-substituted rings, with the exception of those in panels 2 and 5. ND means it was not detected.

It is important that research continue to be done to find many more microorganisms capable of breaking down dioxins and dibenzo furans. Major strides have already been made to identify other types of bacteria and fungi with this capability. *Janibacter* sp. strain YY-1 was isolated from soil by Yamazo, et al. 2004 and was shown to utilize dibenzofuran and fluorene as sole carbon sources. This strain transforms dibenzo-p-dioxin by both angular and lateral dioxygenation pathways. Kimura and Urushigawa also reported this type of transformation for *Rhodococcus opacus* SAO101. Research conducted by Hong, et al.2002 shows biotransformation of 2,7-dichloro- and 1,2,3,4-tetrachlorodibenzo-p-dioxin by *Sphingomonas wittichii* RW1. As the other examples have also shown, Sphingomonas wittichii RW1 can use dibenzofurans and dibenzo-p-dioxins as sole sources of carbon and energy. Fuse, et al.2003 identified three strains of marine bacteria capable of degrading dioxins and dioxin-like compounds. *Alteromonas macleodii*, *Neptunomonas naphthovorans*, and *Cycloclasticus pugetii* were

all shown to have the degradative capabilities. This study was important to show the diversity of bacteria capable of degrading dioxins present in different sources. Alteromonas macleodii was shown to be the most effective of the three marine bacterial strains. The previous examples have shown different bacteria capable of the important degradation of dioxins. Takada, et al. (1996) studied a strain of White Rot Fungus, *Phanerochaete sordida* YK-624, that also possesses these capabilities. Figures 7 and 8 show the effectiveness of P. sordida YK-624 to degrade PCDDs and PCDFs respectively. This fungus and the previously mentioned bacteria are only a few examples of microorganisms with the capability of degrading dioxins and dioxin-like compounds. The above examples demonstrate the plausibility of enzymatic degradation of the dioxin-like contaminants. Additional research is necessary to isolate the appropriate enzymes capable of degrading these compounds and to determine kinetic rates and mechanisms before a feasible remediation scheme could be proposed. It is also important that research continue to be conducted to find new microorganisms capable of degrading these dioxin-like contaminants.

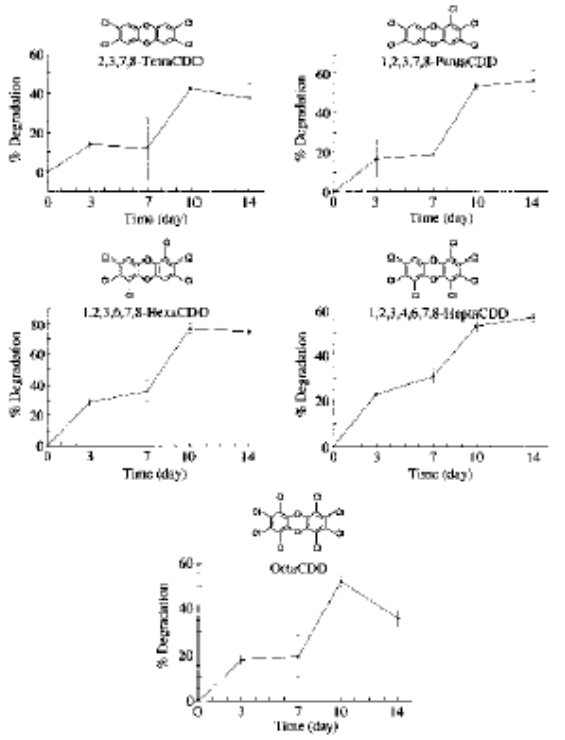

FIG. 7 Degradation of PCDDs by *P. sordida* YK-624.

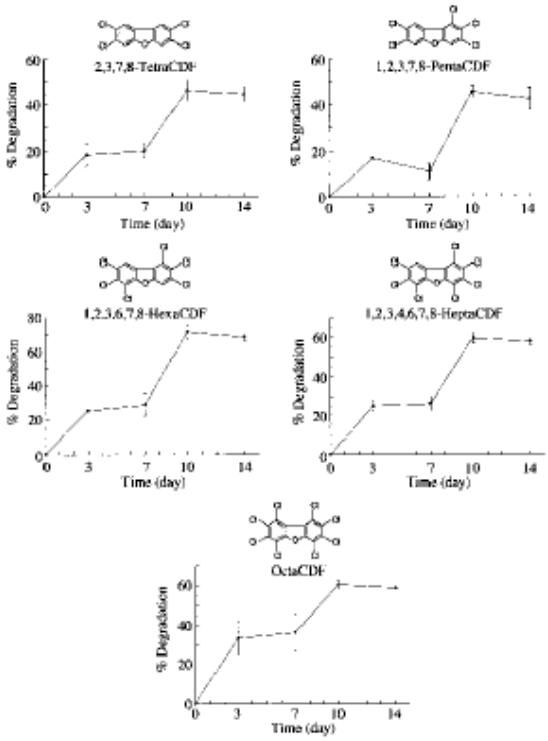

FIG. 8 Degradation of PCDFs by *P. sordida* YK-624.

CONCLUSIONS

While there are many toxic chemicals found in the environment, dioxins have become known as the most toxic, specifically 2,3,7,8-dibenzo-p-dioxin. Studies have shown the numerous harmful effects dioxins and dioxin-like compounds can have on humans. Every year, scientists continue to find more of these adverse effects, stressing the need for the removal of these compounds from the environment.

Since 1995, several in-depth national and international studies have been conducted to measure the concentration and TEQs for dioxin and dioxin-like contaminants in a variety of food categories, including meat, poultry, milk, eggs, fats and oils, fish, and fruits and vegetables; and animal feedstuffs. Parts per trillion TEQs seldom exceed 2, a limit currently adopted in the European community for fats and oils. This limit may be reduced in 2006. Depending on limits promulgated in the United States, there may be the potential for some concern.

It is highly probable that microorganisms are the cheapest, most economically feasible options for this removal. Numerous studies have already shown that bacteria and fungi are valuable and reliable degraders of these highly toxic compounds. This review has highlighted Terrabacter sp. strain DBF63 and Pseudomonas sp. strain CA10 which possess the ability to use their angular dioxygenases to degrade dibenzo-p-dioxins and dibenzofurans.

At present an effective process to remove dioxin-like contaminants from animal coproducts (fats and oils) without degrading the product is unknown. However, a potentially plausible remediation technique centers on the ability of microorganisms to degrade the contaminants. If microorganisms are capable of degrading the contaminants, they must first synthesize the enzymes to catalyze the degradation. Since it is not practical to treat the fats and oils with microorganisms, it is plausible to isolate the cellular enzymes and subsequently add the enzymes to the fats and oils to

achieve enzymatic catalysis. To remove the enzymes from the fats and oils before further processing to edible or inedible products, system temperatures could be elevated to denature the then unwanted enzymes.

It is highly probable that microorganisms are the cheapest, most economically feasible options for this removal. Numerous studies have already shown that bacteria and fungi are valuable and reliable degraders of these highly toxic compounds. This review has highlighted Terrabacter sp. strain DBF63 and Pseudomonas sp. strain CA10 which possess the ability to use their angular dioxygenases to degrade dibenzo-p-dioxins and dibenzofurans.

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Additional Web Sites Surveyed

http://www.dioxinfacts.org/sources_trends/sources.html

http://www.ejnet.org/dioxin/

http://www.renderers.org/

http://www.nationalby-products.com/