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Growing Selenium-Enriched Tobacco

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Selenium levels in tobacco were successfully increased by growing the plants in soil, with added sodium selenite, or by spraying growing plants with an aqueous solution of the chemical. Plants were treated at 4-32 mg of selenium/plant levels. Plant treatments were 5-10 times more effective in increasing selenium levels than soil treatments. Thus, soil treatments at 32 mg/plant increased selenium contents of tobacco leaf lamina to about 2 ppm, while a single plant treatment at the 0.5-mg level produced tobacco with about 0.7 ppm of selenium. Pyrolyses of the cured tobacco showed that about 45% of the selenium could be transferred to tobacco smoke. The rationale for moderate increases in selenium levels in our tobacco smoking products is presented and discussed.

We have initiated research to increase the selenium (Se) content of tobacco as a possible way of reducing the hazards of smoking. This concept is based on the following findings. Twenty years ago, Se compounds were regarded not only as toxic contaminants in food and feed that adversely effected animals in seleniferous regions but also as possible carcinogens. This was the case for Se occurring in both large and small concentrations. Se is toxic at high concentrations but is now regarded as an essential trace element in food and feed. New data are being accumulated on the beneficial effects of trace concentrations of Se in our environment, indicating that Se has important metabolic functions (Harr, 1978) and possesses important protective characteristics. Results in numerous animal studies have indicated that Se significantly inhibited both carcinogens and mutagens (Wattenberg, 1978; Thompson and Becci, 1980; Jacobs, 1980; Adams et al., 1980; Schrauzer, 1976). Also, it was recently shown that low lung cancer countries had 3 times as much Se in their cigarettes as in U.S. cigarettes (Bogden et al., 1980, 1981). However, concentrations of possible tobacco smoke carcinogens, such as tar, nicotine, and polonium-210, were

similar in tobaccos of both high- and low-incidence countries (Bogden et al., 1981). Therefore, since Se may act as an anticarcinogen in humans, it may be beneficial to increase the Se contents of tobacco products. Our initial efforts in growing some flue-cured tobacco with increased Se contents and then determining the transfer of Se to pyrolyzates are presented.

EXPERIMENTAL SECTION

Soil Treatment. In 1981 and 2 weeks after being transplanted, young tobacco plants (variety NC 95) were fortified with solid sodium selenite. The chemical was sprinkled around each plant (four plants per treatment) in a radius of 5 in. and was incorporated into the soil with a garden rake to a depth of 2-3 in. Sodium selenite was applied at three rates: 17.5, 35.0, and 70.0 mg/plant, equivalent to 8, 16, and 32 mg of Se/plant, respectively. The plants were allowed to mature under normal conditions in the field and harvesting of mature leaves was begun 3 months after the first application. The tobacco was cured in the barn under standard conditions and then allowed to air-dry in the laboratory for 24 h. The leaf laminae were separated from the midribs and ground to pass through a 16-mesh sieve. The ground tobacco was then further dried in an oven (95 °C, 24 h) prior to Se analyses or conditioned at 60% relative humidity prior to pyrolysis. The experiment was repeated in 1982, but this time aqueous solutions of sodium selenite were sprayed onto the soil around the plants at rates of 3, 6, 12, and 24

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mg of Se/plant. The tobacco was grown, harvested, and treated as before. Control tobacco was also grown. In the second season, samples of stems, stalks, and soil were saved for Se analyses.

Plant Treatment. Growing tobacco plants were sprayed 3 times with calculated quantities of aqueous sodium selenite solution (219 mg/2.5 L, equivalent to 40 mg of Se/L). Sprayings were begun 6 weeks after transplanting and consisted of three equal treatments at intervals of 2 weeks. Four groups of five plants each were treated and the total amounts of Se applied per plant were 4, 8, 16, and 32 mg. Thus, for example, the 4-mg rate required three sprayings each of 166 mL of the sodium selenite solution, applied evenly to the five plants. Rainfall between the first treatment and final harvesting was recorded. Between the first and second application, 2.8 in. of rain had fallen. Between the second and third application, 0.40 in. of rain was recorded, while between the third application and completion of harvest 7.58 in. of rainfall was measured. The tobacco was cured and treated as above. The plant treatment was also repeated in 1982. In a similar manner, five groups of five plants were treated at the following rates: 0.5, 1, 2, 4, and 8 mg of Se/plant. Rainfall between the first and second treatment was 4.2 in. and 1.6 in. between the second and third application. Between the last treatment and completion of harvest, another 8.7 in. of rain had fallen. The harvested tobacco was treated as before and samples of stems, stalks, and roots were retained for analyses. In an additional experiment, groups of only two plants were subjected to a single spray treatment of 0.5, 1, 4, and 8 mg of Se/plant, at the time of the third plant spray treatment for the above group (3 weeks before first harvest).

Se Analyses. Selenium analyses on the dried tobacco samples were performed by an atomic absorption spectrophotometry method (Ihnat and Miller, 1977). The only modification was the overnight digestion of 1-g samples in nitric acid only, followed by addition of the perchloric acid the next day. Four or three harvestings (primings) were conducted and the leaves from each priming and for each treatment were kept apart. The midribs were removed and the leaf laminae for each priming were combined for analyses. The total sample for a particular treatment was a composite of the primings, in proportion to their weight percentages. The tabulated Se values represent averages of two or three determinations, where low ppm values had relative percent SD of 10–20%, while the high values had relative percent SD of about 10%.

Pyrolysis. Samples (30 g) were pyrolyzed in a moving furnace apparatus and under conditions previously described (Schlotzhauser et al., 1979). Pyrolysis products were collected in traps containing chloroform-methanol (9:1 v/v). The pyrolyzate solutions were concentrated under vacuum on rotary evaporators (30 °C) to a small volume and diluted to 25.00 mL. Aliquots (10–15 mL) of the pyrolyzate stock solutions were submitted for Se analyses or concentrated to dryness for pyrolysis yield determinations.

RESULTS AND DISCUSSION

The Se contents of American cigarettes, containing mostly American tobaccos, have been given as 0.12–0.31 µg/g or ppm (Bogden et al., 1981). The Se content of Pakistani cigarette tobaccos was found to vary between 0.11 and 3.15 ppm (Ahmad et al., 1979). Our Se analyses on various flue-cured and burley tobaccos gave values in the 0.02–0.09-ppm range. The mean Se content in ciga-

Table I. Se Contents of Cured Tobacco Leaf Lamina Resulting from Soil Treatment (1981 Crop)^a

	ppm of Se for mg of Se added to soil		
	8 mg	16 mg	32 mg
priming			
(harvesting): A	0.20	0.38	1.33
B	0.43	0.40	1.22
C	0.46	0.55	1.57
D	0.83	0.73	2.90
mixture (by wt %)	0.69	0.63	1.86

^a Untreated control tobacco had a 0.06 ppm of Se content.

Table II. Se Contents of Cured Tobacco Leaf Laminae, Leaf Stems, and Stalk Resulting from Soil Treatment (1982 Crop)^a

	ppm of Se for mg of Se added to soil			
	3 mg	6 mg	12 mg	24 mg
laminae				
priming				
(harvesting): 1	0.05	0.18	0.35	0.32
2	<0.01	0.05	0.11	0.13
3	<0.01	0.01	0.03	0.08
mixture (by wt %)	<0.01	0.04	0.10	0.15
stem (mixture)	<0.01	<0.01	0.07	0.07
stalk	<0.01	0.05	0.03	0.05
roots		0.09	0.21	0.14
soil ^b (surface)	— ^c	0.09	0.11	0.12
soil (8-in. depth)	—	0.10	0.14	0.22

^a Untreated control tobacco (stems, stalks, laminae) had 0.01 ppm of Se content or less. ^b Untreated soil gave a value of 0.06 ppm of Se. ^c Not determined.

rettes from countries with a high lung cancer rate (United States, Britain, Sweden) was determined to be about 0.16 ppm, while that of cigarettes from the low-incidence countries averaged about 0.40 ppm (Bogden et al., 1981). Thus, it may be beneficial to increase the Se content of all tobaccos, especially U.S. and British tobaccos, to the 0.40-ppm level, or more.

Since the only forms of Se that are taken up readily by plants are selenite, selenate, or organic forms (Frost and Lish, 1975), it seemed easiest to fortify the soil with water-soluble sodium selenite. The quantities of Se to be applied were based on three considerations. Soils generally contain about 0.1 ppm of water-soluble Se (Ganje, 1973). The seleniferous soils in the United States are found in the Western region, from North Dakota to Texas and west to the Pacific Coast, while the soils in the Eastern United States, where most of the tobacco is grown, are regarded as Se deficient (Frost and List, 1975). The soil in the portion of the field where the control tobacco was grown was later shown to contain 0.06 ppm of Se. Also, since most plants are believed to take up very low quantities of selenite from the soil (Ganje, 1973), it was decided to add relatively large amounts of sodium selenite to the soil around the plants. This resulted in substantial increases in Se in the laminae of the 1981 flue-cured tobacco leaf (Table I). For the 1982 crop, relatively lower values were obtained (Table II). This was probably due to use of an aqueous solution of sodium selenite (instead of solid chemical) that was more uniformly distributed and was probably washed away to a greater extent by the heavier rainfalls of 1982, which was regarded as a very wet season. The movement of Se away from the roots is indicated by

Table III. Se Contents of Cured Tobacco Leaf Laminae Resulting from Plant Treatment (1981 Crop)^a

	ppm of Se for mg of Se sprayed on plant			
	4 mg	8 mg	16 mg	32 mg
priming				
(harvesting): A	2.87	6.22	14.55	22.30
B	3.62	5.63	7.54	7.47
C	1.74	2.77	5.42	7.75
D	1.25	2.57	4.47	6.10
mixture (by wt %)	2.54	3.73	7.01	9.38

^a Untreated control tobacco had a 0.06 ppm of Se content.

somewhat higher levels of Se in the soil at the 8-in. depth compared to those at the surface (Table II).

Since cigarette tobacco is mainly composed of leaf laminae, the midribs were separated from the laminae. In 1981, only the laminae were analyzed. The laminae from the 8-mg soil treatment were shown to contain about 0.7 ppm of Se, a 10-fold increase in Se content compared to the control tobacco sample. The high application of 32 mg/plant produced tobacco with a 1.9 ppm of Se content. For 1981, analyses of individual primings (harvesting) showed that the last priming (upper leaves) had higher values than the first priming (A). This trend was not found for the 1982 tobacco, which had absorbed much less of the applied Se. It was concluded that, although the tobacco plants did take up sufficient Se to increase their content up to 30-fold, fortification of soil with the chemical would not be an economical method. Also, the application of the chemical around the plant probably did not produce a very uniform concentration in the soil (Table III). Calculations showed that only 0.1–2% of the applied Se was absorbed by the plants.

Another series of plants was subjected to a spraying treatment with aqueous solutions of sodium selenite. In 1981, plants were sprayed 3 times during their growing period to yield total plant treatments of 4, 8, 16, and 32 mg of applied Se/plant. Mature leaves were harvested and flue-cured in the traditional manner and only the leaf laminae were analyzed. The 4 mg/plant treatment produced tobacco with a 2.5 ppm of Se content, while the highest 32 mg of Se/plant spraying gave a 9.4 ppm of Se tobacco (Table III). A comparison of the values at the same application level showed that the plant sprayings were about 5 times more effective. When the values for the individual primings (harvesting) are compared, the first priming (A, lowest leaves) had a much higher Se content than the last priming (D, top leaves). It may be that the lower leaves received more Se per area of leaf in the course of the three treatments, as they were more developed than the growing upper leaves. Also, any rainfall after a treatment would wash the Se from the upper leaves onto

the lower leaves. The question of whether the Se was absorbed into the leaf or remained on the leaf as a residue could not be definitely answered. Sodium selenite is highly water soluble and probably did not remain as a residue on the leaves, as there was 8 in. of rainfall between the last treatment and the last priming. Some absorption of Se into the leaves must have occurred. Lack of translocation into the upper leaves may be due to binding of Se to some leaf constituent, such as a protein, similar to its proposed binding to proteins in tumor cells (Schwartz, 1975).

The plant applications were also repeated the following year at 0.5–12-mg rates (Table IV). This time resulting Se contents were more comparable than for the soil treatments. The rate of 8 mg of Se/plant produced 3.73 and 5.02 ppm for 1981 and 1982, respectively. Thus, in spite of heavier 1982 rains, similar quantities of Se were absorbed and retained. The same trend in primings was also observed, with the last priming (top leaves) having the lowest values. From the values for the stems (Table IV), there is an indication of translocation of Se. If only absorption was involved, the ratio of the area of the stems relative to the area of the laminae would indicate that much smaller quantities of Se would adhere to the stems. But as the values for the stems were relatively high, some translocation may have occurred. The higher than normal values for the roots probably indicate absorption of Se from the soil of the chemical that was washed off by the rain.

The percentages of absorbed Se in the 1981 plant treatments were calculated (Table V). The 4-mg treatment resulted in a 10% absorption, while only 7% of the 32 mg of Se was absorbed. From the 1981 data, it appeared that spraying tobacco plants with 1 mg or less of Se/plant should produce tobacco with a 0.5 ppm of Se content. Such an application may be easily made in the field together with the spraying of the sucker control chemicals. This one-time application would probably suffice and would not require any additional labor. This idea was examined by a single-treatment experiment in 1982. Plants were sprayed once, about 3 weeks before the first priming (harvest), at rates of 0.5–8 mg of Se/plant (Table VI). It was found that a 1-mg treatment yielded laminae with a 1.7 ppm of Se content, while the 0.5-mg treatment gave laminae with 0.75 ppm of Se. Consequently, an application of about 0.3 mg of Se/plant, together with the sucker control treatment, should suffice to produce tobacco with the desired 0.5 ppm of Se content.

It was of interest to us to examine the fate of the increased Se in the tobacco in order to answer the question as to how much could be transferred into tobacco smoke. Since sufficient tobacco was not available to manufacture cigarettes, we resorted to our standard method of pyrolysis (thermal degradation). Pyrolyses of the tobacco samples were conducted under conditions that produce chroma-

Table IV. Se Contents of Cured Tobacco Leaf Laminae, Leaf Stems, and Stalk Resulting from Triple Plant Treatment (1982 Crop)^a

		ppm of Se for mg of Se sprayed on plant (3×)					
		0.5 mg	1 mg	2 mg	4 mg	8 mg	12 mg
laminae							
priming (harvesting):	1	1.52	0.41	1.52	4.29	9.73	12.6
	2	0.37	0.28	0.36	2.01	6.03	7.94
	3	0.20	0.12	0.12	0.39	2.11	2.71
mixture (by wt %)		0.59	0.28	0.46	1.73	5.02	8.03
stem (mixture)		0.06	0.14	0.14	0.39	1.32	2.14
stalk		0.18	<0.01	<0.01	0.13	0.47	0.31
roots		^b	–	–	0.10	0.18	0.62
soil (8-in. depth)		–	–	–	–	0.13	0.15

^a Untreated control tobacco had a 0.01 ppm of Se content. ^b Not determined.

Table V. Percentage of Se Absorbed—1981 Plant Treatment

applied Se, mg/plant	4	8	16	32
total cured tobacco, g ^a	836	956	767	1121
Se content, ppm ^b	2.54	3.73	7.01	9.38
total Se in tobacco, mg ^b	2.12	3.57	5.38	10.51
total Se applied, mg ^c	20	40	80	160
% of applied Se in laminae ^d	10.3	8.8	6.7	6.5

^a Total weight of lamina from five plants. ^b In laminae only, excluding stalk and stems. ^c To five plants. ^d Corrected for a native Se level of 0.06 ppm in laminae.

Table VI. Se Contents of Cured Tobacco Leaf Laminae, Leaf Stems, and Stalk Resulting from Single Plant Treatment (1982 Crop)^a

	ppm of Se for mg of Se sprayed on plant (1×)			
	0.5 mg	1 mg	4 mg	8 mg
laminae priming				
(harvesting): 1	2.90	4.51	6.13	9.85
2	0.77	1.63	2.54	6.01
3	0.22	0.37	0.45	2.05
mixture (by wt %)	0.75	1.76	3.00	5.94
stem (mixture)	0.15	0.34	0.65	1.64
stalk	0.02	0.07	0.13	0.27
roots		0.085	0.21	0.37

^a Untreated control tobacco had a <0.01 ppm of Se content.

Table VII. Transfer of Se from Tobacco to Pyrolyzate

tobacco		pyrolyzate			transfer, %
Se in sample, ppm	total Se, ^a μg	weight, g	ppm of Se	total Se, ^b μg	
0.83	20.7	4.75	1.54	7.3	35
0.73	14.4	4.35	1.78	7.7	54
2.90	55.8	5.32	5.40	28.7	52
2.54	76.2	4.62	5.18	23.9	32
3.73	111.9	4.93	10.42	51.4	46
7.01	210.3	5.46	20.8	113.8	54
9.38	281.4	5.45	23.1	125.6	45

^a In 30-g sample. ^b In total pyrolyzate from a 30-g sample (weight × ppm).

topographic profiles and product distributions identical with those of cigarette smoke (Schlotzhauer et al., 1979). Pyrolyzates, which are similar to cigarette smoke tar, were analyzed to determine the quantities of volatilized Se (Table VII). The percentage transferred from the fortified tobaccos to the pyrolyzates varied from 32 to 54%, for an average transfer rate of 45%. Apparently, the total quantity of Se present did not effect the rate, as similar quantities of Se were volatilized for both low- and high-content tobaccos. The 45% transfer rate indicates that about 55% of the Se was retained in the charred pyrolysis residue or ashes. This value is reasonably close to a report stating that 45% of the Se in a cigarette was retained in the ash and that the volatilized Se was not retained by the filter (Ahmad et al., 1979). This means that about half of the Se in the tobacco would be volatilized into the sidestream smoke and mainstream smoke of a cigarette, with the smoker inhaling about 15% of the total Se in the cigarette tobacco (about one-third of the volatilized Se will be in the inhaled mainstream smoke). Thus, a pack of cigarettes, made of 0.5 ppm of Se tobacco, would yield mainstream smoke containing about 1 μg of Se. This is a negligible amount, in view of the fact that the human daily (food) intake is 60–150 μg and the human body

burden is about 15 000 μg/70 kg (Doull et al., 1980). Also, the toxic chronic dose of Se for human beings, borderline for overt signs of Se poisoning, is about 2400–3000 μg of Se/day for a long period of time (Wilber, 1980). For dietary supplementation, the Se intake has been suggested at 50–200 μg/day (Hubbard et al., 1980).

These facts raise the interesting question as to the possibly protective action of Se in the lungs of the smoker. Little is known of this antineoplastic action, but Se seems to have many roles in mammalian metabolism. It is involved in the biosynthesis of ubiquinone (coenzyme Q), is a part of glutathione peroxidase, and controls lipid peroxide formation (Doull et al., 1980). Thus, it may be that the usual concentration of Se in lung tissue is not sufficient to deter tumor initiation by smoke carcinogens, such as the polynuclear aromatic hydrocarbons. But, when increased quantities are inhaled together with the smoke carcinogens, then the antineoplastic effect of Se should become apparent, similar to its effect on benzo[a]pyrene- and benzanthracene-induced skin tumors in mice and mammary carcinogenesis in rats (Welsch et al., 1981). Also, Se may aid in detoxifying cigarette smoke by removal (precipitation and retention on cigarette or filter) of such toxic trace metals as nickel, cadmium, copper, and arsenic, as their insoluble selenides (Harr, 1978). These trace metals occur in significant concentrations (Cu, 0.19, Cd, 0.12, As, 0.12, and Ni, 0.08 μg/cigarette) in cigarette smoke ("Smoking and Health—A Report of the Surgeon General", 1979).

It was concluded that Se concentrations of tobacco can be readily increased. Single or multiple Se applications as foliar sprays during the growing stage of the tobacco plant are more effective and economical than soil treatments at the transplanting stage. From the data, it appears possible to produce tobacco with any desired Se content. A 3–10-fold increase above the normal Se levels of American tobacco may prove beneficial. On the basis of reports in the literature, this may result in a less harmful smoking product for the 50 million Americans who continue to smoke. It will be the task of biological assays to determine if increases in Se concentration would give a safer smoking product.

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Registry No. Selenium, 7782-49-2; sodium selenite, 10102-18-8.

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Synthesis and Characterization of Twenty-two Purified Polychlorinated Dibenzofuran Congeners

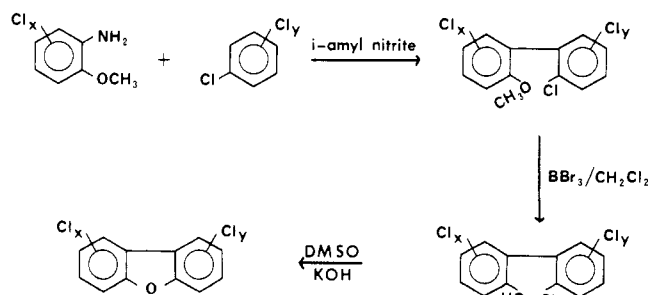
Stephen H. Safe* and Lorna M. Safe

The identification of toxic polychlorinated dibenzofurans (PCDFs) in diverse environmental matrices requires the availability of purified standards for analytical, toxic, and biologic studies. This study reports the synthesis and characterization of 22 purified PCDF congeners by the base-catalyzed cyclization of their corresponding hydroxypolychlorinated biphenyl (PCB) precursors containing *o*-chloro and -hydroxy substituents on the two phenyl rings. The synthesis of the hydroxy PCBs (from their methoxy analogues) was accomplished by using two main routes, namely, (1) the diazo coupling of chlorinated anisidines and symmetrical chlorinated benzenes and (2) the diazo coupling of chlorinated anilines and chlorinated anisoles. By the judicious selection of the synthetic precursors it is conceivable that these schemes could be used to prepare most of the PCDF congeners.

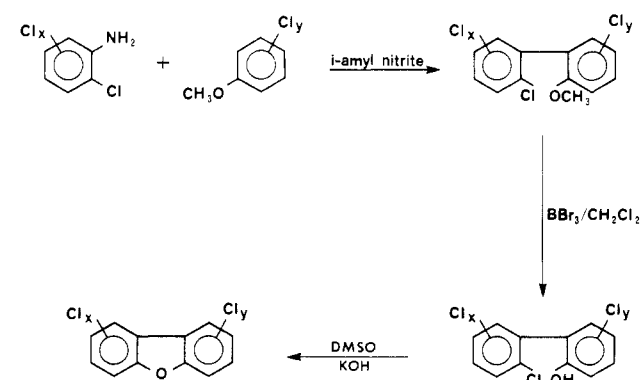
Halogenated aromatic hydrocarbons are among the most widespread and persistent environmental contaminants (Landrigan, 1980; Safe, 1982). Included in this group of structurally related compounds are the polychlorinated biphenyls (PCBs), naphthalenes (PCNs), polybrominated biphenyls (PBBs), polychlorinated dibenzofurans (PCDFs), and dibenzo-*p*-dioxins (PCDDs). The PCDDs and PCDFs are not primary industrial products but are formed as impurities in chlorinated phenol, PCB, and PCN formulations (Buser and Bosshardt, 1976; Blaser et al., 1976; Buser, 1975; Levin and Nilsson, 1977; Nilsson and Renberg, 1974; Firestone, 1977; Rappe et al., 1978a; Norstrom et al., 1979; Vos et al., 1970; Buser et al., 1978c; Bowes et al., 1975) or are formed as byproducts in diverse combustion processes (Olie et al., 1977; Lustenhouwer et al., 1980; Ahling et al., 1977; Buser et al., 1978b,c; Buser, 1979; Lindahl et al., 1980; Kooke et al., 1981; Hutzinger et al., 1980; Eiceman et al., 1979; Nestrick et al., 1982; Gizzi et al., 1982; Choudhry and Hutzinger, 1982; Buser and Rappe, 1978, 1979). PCDDs and PCDFs are the most toxic halogenated aromatic pollutants, and it has been shown that although there are 75 and 135 possible PCDD and PCDF isomer congeners, only a limited number of these compounds are toxic (Poland and Knutson, 1982). The most active individual compounds within each class are approximate isostereomers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF); however, the precise number of toxic congeners and their relative activities has not been fully delineated.

Analysis and quantitation of PCDFs in the environment are complicated by the lack of analytical standards available. There are several synthetic routes that have been utilized for the synthesis of specific PCDF congeners (Gara et al., 1981) and these include the following: (1)

Scheme I. Synthesis of Purified Polychlorinated Dibenzofurans



Scheme II. Synthesis of Purified Polychlorinated Dibenzofurans



chlorination of dibenzofuran and its chlorinated homologues; (2) the reduction of chlorinated *o*-nitrodiphenyl ethers followed by internal diazo coupling (Gray et al., 1976); (3) internal cyclization of *o*-chloro-substituted diphenyl ethers by chemical or photolytic techniques (Choudhry et al., 1977; Norstrom et al., 1976, 1977; Gara et al., 1979); (4) pyrolysis of PCBs, chlorinated phenols and derived products, and chlorinated diphenyl ethers (Ahling

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