Etiology of Chick Edema Disease

by David Firestone*

Chick edema disease first came to the attention of the Food and Drug Administration in December, 1957, when it was learned that millions of broilers died in the eastern and midwestern parts of the United States. Several groups in industry and government quickly determined that the disease was due to toxic components in certain feed fats (*1–3*), and that toxicity was associated with the unsaponifiable portion of the fat.

Characteristic symptoms included the presence of excessive fluid in the heart sac and in the abdominal cavity of chicks fed toxic fat (*2, 4, 5*). These and other symptoms such as subcutaneous edema and liver necrosis were accompanied by high mortality beginning approximately in the third week. Allen (*6, 7*) suggested several years later that the accumulation of large quantities of extravascular fluid in chickens might be due to altered permeability of the vascular bed as well as cardiac decompensation and liver necrosis.

Developments Prior to Chemical Identification of the Toxic Factors

Initial outbreaks of the disease in 1957 occurred at a time of increased demands by feed manufacturers for low-cost fats to raise the caloric level of diets for food animals. Investigations by the Food and Drug Administration in 1958 soon demonstrated that chick edema disease was caused by toxic material in by-product fatty acids obtained from production of oleic and stearic acids added to certain lots of feed-grade fats. Further investigation showed that toxic material was also present in various distillates and still residues obtained from several fatty acid producers who prepared commercial oleic and stearic acids from inedible tallow (*8*). The most toxic samples were batch still distillates obtained after repeated distillation of tallow fatty acids. The general scheme used for production of commercial fatty acids is shown in Figure 1. Fatty acids

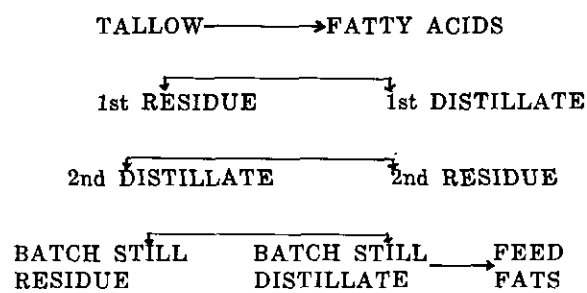

FIGURE 1. Scheme for commercial production of fatty acids and by-product (batch still) distillate from tallow.

obtained from hydrolysis of tallow were distilled in continuous stills to produce first and second (continuous still) distillates which were subjected to further processing to produce oleic and stearic acids. The second (continuous still) residue was then distilled in a batch still to yield by-product (batch still distillate) fatty acids which were sold as feed fats. Occasional vegetable oil fatty acid samples were also found to exhibit some chick edema activity (*8*), but it could not be determined whether these samples

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were inherently toxic or were made toxic by cross contamination in the plant with toxic tallows or toxic tallow acids.

Now that it was demonstrated that chick edema disease was caused by toxic substances present in the feed, the possibility existed that edible chicken flesh might contain these toxic materials. The unsaponifiables isolated from the carcasses of chickens fed toxic fat were fed to chicks at various levels (0.025–1.0%) in a test ration (*2*). Symptoms of edema disease were observed at all levels fed. The unsaponifiables from normal control chickens, fed to chicks at 0.5% of the diet produced no abnormal symptoms in test checks. Similarly, the presence of toxic factor was demonstrated in the meat of hogs that had been fed toxic fat (*9*).

The presence of toxic factor in commercial still distillates and residues prompted the examination of oleic acids and stearic acids collected in 1959 from food manufacturing plants. Oleic acids from several plants showed varying degrees of chick edema toxicity (*8*). In addition, oleic acid derivatives such as triolein and glyceryl monooleate were found to be toxic. Ames et al. (*10*) also found chick edema factor in a number of oleic acids and glyceryl monooleates. No toxicity was found in commercial stearic acids. At this stage, the identity of the toxic factors was unknown, but evidence clearly indicated that they were chlorinated aromatic compounds (*11*).

Since chick edema factor was found in food grade oleic acid and derivatives, the Food and Drug Administration issued a Food Additive Regulation in 1960 for fatty acids (*12*) specifying that they be free of chick edema factor in accordance with a 3-week chick feeding bioassay. The need for a rapid screening test was met by development of a microcoulometric gas chromatographic method (*13*) involving cleanup of isolated unsaponifiables by adsorption chromatography on activated alumina prior to gas chromatographic analysis. Portions of a reference toxic fat obtained in 1958 were made available as positive standards.

The availability of methodology assisted in bringing the chick edema disease under control after another outbreak in the Southeast in 1960. Although the structures of the substances causing the disease were yet to be determined, the typical pattern of gas chromatographic peaks with long retention times versus aldrin (Fig. 2) was found useful for detecting toxic fats.

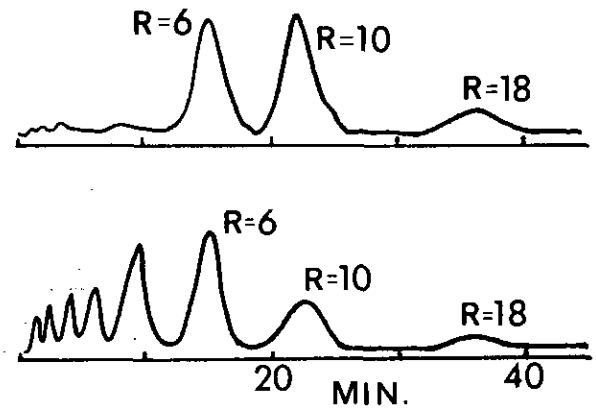

FIGURE 2. Microcoulometric gas chromatograms of material isolated from two toxic by-product fatty acids obtained from commercial fatty acid manufacturers. GLC column temperature 248°C; *R* denotes retention time versus aldrin.

Subsequently, electron capture–gas chromatographic procedures were developed (*14–17*) which were approximately 2000 times more sensitive than the microcoulometric methods and could detect less than 10 ppb of chick edema factors in lipid samples. Electron-capture gas chromatography also provided increased resolution so that additional peaks with characteristic retention times could be observed in extracts from toxic fats (see Fig. 3). In addition, a bioassay test with increased sensitivity was developed (*18,19*) using fertile chick eggs. Injection of chick edema factors resulted in decreased hatch and development of embryonic deformities and edema (*19*). Embryos which failed to hatch exhibited malformed beaks, eye defects, leg deformities, and lack of development of the right mesencephalon. Embryos which hatched exhibited

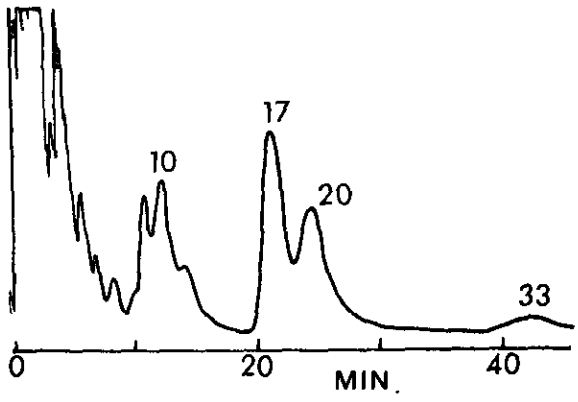

FIGURE 3. Electron-capture gas chromatogram of extract from a toxic commercial glyceryl monooleate, GLC column temperature, 210° C; numbers above peaks are retention times versus aldrin.

sparse and defective feathering and growth retardation.

Identity of the Chick Edema Factors

Chromatographic behavior and ultraviolet absorption spectra of toxic fractions indicated initially (*2*) that the toxic factors were substituted aromatic compounds, perhaps substituted naphthalenes or phenanthrenes or aromatic steroids. A major breakthrough on the arduous road to identifying the structure of chick edema factors occurred in 1960 when Harmon et al. (*11, 20*) isolated crystalline material from a toxic fat and determined that it was an aromatic substance containing 47% chlorine (M. Tishler, Merck and Co., private communication). A similar crystalline material which was chick edema-active at 0.1 ppm in the diet was isolated from a sample of commercial triolein found to be toxic to Cebus monkeys (*20*). The monkeys fed the toxic triolein exhibited fatty liver, liver necrosis, pancreatic atrophy and fibrosis, bile duct proliferation, hemosiderosis, gross hemorrhage in the gastro-intestinal tract, and erythrocytophagocytosis. Subsequently, Wootton and Courchene (*21*) characterized two compounds isolated from a toxic feed fat as hexachlorohexahydrophenanthrenes on the basis of mass and other spectral data.

Finally, in 1966, Cantrell et al. (*22*) showed by x-ray crystallography that one of the active crystalline materials isolated earlier from toxic fat was 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin. Wootton immediately demonstrated that a synthetic hexachlorinated dibenzo-*p*-dioxin displayed properties similar to the material isolated from the toxic fat and, in addition, produced the chick edema disease in chickens (J.C. Wootton, Procter and Gamble Co., private communication, 1966).

Formation and Occurrence of Chlorodibenzo-*p*-dioxins (Dioxins)

Tomita et al. (*23*) had shown that chlorophenols and their salts can condense to form chloro derivatives of dibenzo-*p*-dioxin. Thus, chlorophenols appeared to be the source of dioxins. Accordingly, FDA scientists (*24*) pyrolyzed a number of commercially available chlorophenols, including pentachlorophenol, and obtained chlorodioxin mixtures with GLC peaks having retention times identical to those found in toxic fats. The most toxic pyrolysis product, identified as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, was obtained from 2,4,5-trichlorophenol. Hexa-, hepta-, and octachlorodioxins were obtained from pentachlorophenol. Individual components were isolated by preparative GLC from a reference toxic fat. Dioxins with 3, 4, 6, and 7 chlorine atoms were toxic to chick embryos. The presence of 2,3,7-trichloro- and 2,3,7,8-tetrachlorodioxin as well as hexa-, hepta-, and octachlorodioxins in the reference toxic fat indicated that the tallow from which the fat was derived was contaminated with 2,4,5-trichlorophenol as well as pentachlorophenol. Kimmig and Schulz (*25*) demonstrated in 1957 that 2,3,7,8-tetrachlorodioxin was highly toxic and chloracnegenic and was formed in the industrial production of 2,4,5-trichlorophenol by alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene. Toxicological studies of individual chlorodioxins with chicks (*26*) and chick

embryos (M. J. Verrett, private communication, 1970) again showed that the 2,3,7,8-tetrachlorodioxin was the most toxic. Hexachlorodioxins were about one fifth as toxic and 2,7-di- and octachlorodioxins were the least toxic.

Pentachlorophenol and other chlorophenols have been widely used as bactericides, slimicides, defoliants, and termite control agents in industry and agriculture. They are also used in the manufacture of food-packaging materials (Subpart E, Title 21 of the Code of Federal Regulations (21 CFR), Part 121 § 121.2001) and as components of articles that contact food (Subpart F, 21 CFR, §121.2500). A list of food additive uses of 2,4,5-trichloro- and pentachlorophenol is given in Table 1. Because of their widespread use, a variety of commercial chlorophenols were examined for the presence of individual dioxins and related compounds (*27*) Nonacidic material isolated from the chlorophenols was chromatographed on alumina, and fractions were examined by combined gas chromatography–mass spectrometry.

Table 1. Food additive uses for 2,4,5-trichloro- and pentachlorophenol.[a]

Regulation No.	Specific compound[b]	Use
Subpart E:		
121.2001	TCP–Na; TCP–K; PCP–K	Slime control in manufacture of paper and paperboard
Subpart F:		
121.2505	TCP–Na; PCP–Na	Slimicides in manufacture of paper and paperboard
121.2514 (b) (3) (xxxi)	PCP–Na	Preservation in can end cements
121.2519 (d) (3)	PCP–Na; TCP–Na	Defoaming agents in manufacture of paper and paperboard
121.2526 (5)	PCP–Na	Paper in contact with aqueous and fatty foods
121.2534 (d) (3)	PCP–Na	Animal glue for articles holding food
121.2550 (b) (5)	PCP–Na; PCP–K	Closures with sealing gaskets for food containers
121.2556 (b)	PCP; PCP–Na	Wood preservative
121.2557 (d) (3)	TCP–Na; PCP–Na	Preservative for defoaming agents used in coatings
121.2562 (c) (4) (iii)	PCP–Na	Antioxidant in rubber articles used in producing, processing, or holding food
121.2596	PCP–Na	Preservative for ammonium alginate used in manufacture of poly(vinyl chloride) polymers that contact food

[a] Title 21, Code of Federal Regulations.
[b] TCP–Na=*sodium trichlorophenate*; TCP–K=*potassium trichlorophenate*; PCP=*pentachlorophenol*; PCP–Na=sodium pentachlorophenate; PCP–K=potassium pentachlorophenate.

A variety of polychlorodibenzofurans (furans) and polychlorodiphenyl ethers (ethers) was found in the chlorophenols in addition to dioxins. Up to 6.2 ppm 2,3,7,8-tetrachlorodioxin was found in six samples of 2,4,5-trichlorophenol, and up to 39 ppm hexachlorodioxins was found in eight samples of pentachlorophenol. In addition, chloromethoxyfurans and chloromethoxyethers were found in 2,4,5-trichlorophenol, and chlorohydroxybiphenyls were found in pentachlorophenol.

Chlorodioxins, furans, and ethers found in commercial pentachlorophenols are indicated in Table 2. Combined gas chromatography–mass spectrometry (GC–MS) was used to detect mixtures of these compounds which were unresolved by GLC. Characteristic mass spectral peaks for a dioxin, a furan, and an ether are shown in Table 3. The

Table 2. Dioxins, furans, and ethers in commercial pentachlorophenols.

No. of Cl atoms	Detected by combined GC–MS		
	Dioxins [a]	Furans [b]	Ethers [c]
4		+	+
5		+	+
6	+	+	+
7	+	+	+
8	+	+	+
9			+
10			+

[a] Chlorodibenzo-*p*-dioxins.
[b] Chlorodibenzofurans.
[c] Chlorodiphenyl ethers.

molecular weight and fragmentation patterns of the dioxins, furans, and ethers were sufficiently different so that unresolved mixtures were readily characterized by combined GC–MS. The results of analysis of the commercial chlorophenols indicated that the outbreaks of chick edema disease could have originated from tallows contaminated with chlorophenols containing preformed dioxins.

Table 3. Characteristic mass spectral fragments of dioxins, furans, and ethers.

Fragment	Relative intensity, % [a]		
	Cl_4–Dioxin [b]	Cl_4–Furan [c]	Cl_5–Ether [c]
M	78	72	22
M–Cl	7	10	2
M–2Cl	3	15	54
M– (CO + Cl)	35	50	—
M–2(CO + Cl)	25	—	—
M– (CO + 2 Cl)	—	15	—
M– (CO + 3 Cl)	—	75	8

[a] Based on ^{35}Cl; 1% or greater relative intensity.
[b] 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin.
[c] Positions of chlorine substitution unknown.

Probable Source of Chick Edema Factors

Reports in early 1960's indicated that pentachlorophenol was widely used as a preservative in hide stripping operations, and might be present in the by-product tallows (fleshing greases) recovered from hides. A number of papers in leather trades periodicals (*28–30*) described the use of trichloro- and pentachlorophenol as preservatives for hide curing operations. A major domestic manufacturer of chlorophenols recommends the use of the sodium salts of 2,4,5-trichlorophenol or pentachlorophenol for a variety of hide preservation and hide treatment operations as well as for general plant sanitizing procedures (*31*). Pretanning operations include trimming, brining (or salt curing) of the hides, followed by soaking, liming, bating (neutralization with buffering salts and treatment with a proteolytic enzyme), and pickling (*32*). The sodium salt of pentachlorophenol is recommended for use in salt curing, brining, soaking and pickling operations, and the sodium salt of 2,4,5-trichlorophenol is recommended for use in soaking operations (*31*). By-product fats are obtained after trimming, soaking and liming treatments (*32*) so that the use of chlorophenols as preservatives during processing can result in contamination of the hide greases with chlorophenols.

Contaminated hide greases may have been the source of the chick edema outbreaks of 1957 and 1960. Accordingly, three commercial oleic acids examined earlier for chick edema factor were reexamined for the presence of chlorophenols (*33*). Two of the samples that were previously found to be positive for chick edema factor were also found to be contaminated with 2,3,4,6-tetrachlorophenol and pentachlorophenol. The third sample contained a trace of pentachlorophenol.

Finally, Metcalfe (*34*) presented concrete evidence that the source of chick edema factor was fleshing grease from hides that had been treated with commercial pentachlorophenol. He examined samples of fleshing grease from pentachlorophenol-treated hides as well as industrial tallows containing glue emulsion with added pentachlorophenol. Chlorophenol-containing glue emulsions have been used in dry rendering operations. A fleshing grease sample was found to be extremely toxic when tested for chick edema factor (chick feeding bioassay) at the 16% level (*34*). A gas chromatogram of chlorodioxins isolated from this fleshing grease is shown in Figure 4. (The numbers above the

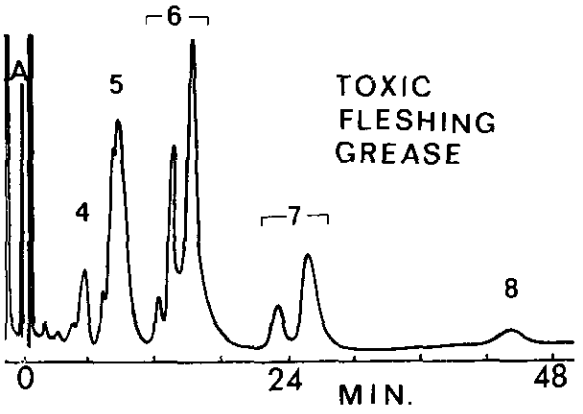

FIGURE 4. Electron-capture gas chromatogram (*34*) of dioxins extracted from a toxic fleshing grease. The numbers above the peaks refer to the number of halogen atoms in the individual dioxin molecules.

GLC peaks refer to the number of halogen atoms in the individual dioxins.) The Division of Chemistry and Physics, Food and Drug Administration, assisted with analysis of the sample. The presence of tetra-, penta-, hexa-, hepta-, and octachlorodioxins was confirmed by combined GC–MS. Penta- and hexachlorodioxins comprised 70% of the total dioxins found. The widespread use of chlorophenols as hide preservatives prior to the middle 1960's is the probable cause of the earlier outbreaks of chick edema disease. The use of chlorophenol-containing glue emulsions for dry rendering is another possible source of toxic tallows.

In 1970, a survey was made of a selected number of tallow processors and fatty acid producers. Ten of 45 samples of tallow and oleic acid were found to contain up to 77 ppb of dioxins (hexa-, hepta-, and octachlorodioxins). Four non-food-grade oleic acids contained 11 to 77 ppb of dioxins, and one food-grade oleic acid contained 59 ppb of dioxins. Fleshing grease from two hide processors contained low levels (5 and 11 ppb of dioxins), indicating that the use of chlorophenols for hide processing has not been entirely discontinued.

Origin of 1969 Outbreak of Chick Edema Disease

In early 1969, an outbreak of chick edema disease in North Carolina resulted in the death or destruction of some 300,000 chickens. An additional million birds were involved in further outbreaks in the next several weeks. The cause was traced to the use of contaminated vegetable oil by-product fatty acids in the feed.

Investigation by the Food and Drug Administration disclosed that the feed fat became contaminated at a vegetable oil refinery which also formulated antimicrobial water treatment products containing various chlorophenols. An underground pipe line was found leading from the "pesticide" product plant to traps used to collect by-product fatty acids (acidified soapstock) from the vegetable oil refinery. This pipe line unintentionally transferred "pesticide" plant wash water to the traps holding acidified soapstock, resulting in contamination of the acidified soapstock intended for sale as feed fats. The possibility of further contamination was eliminated by removal of the "pesticide" operation from the refinery site.

Prevention of Dioxin Contamination of Foods and Feeds

Since commercial chlorophenols are widely used as termite control and antimicrobial agents, there are numerous opportunities for direct and indirect contamination of food and food fats. Dioxin contamination of fats and fatty acids destined for use in foods or feeds can be minimized by control of sources of direct contamination with chlorophenols. These sources include the use of tri- and pentachlorophenol for hide preservation and other pretanning operations and in glue emulsions for dry rendering of fats. Care must also be taken in the use of chlorophenols industrially (as antimicrobials) or on the farm (as wood preservatives for fences, barns, etc.).

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