

**OCCUPATIONAL HEALTH STUDY OF MIDWESTERN FORMULA FEED MILLS**

by

**Roy L. Gibson, Surgeon  
Mars Y. Longley, S. A. Scientist**

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**Division of Occupational Health  
Public Health Service  
U.S. Department of Health, Education and Welfare  
1014 Broadway, Cincinnati 2, Ohio**

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## NATURE & SCOPE OF STUDY

The formula feeds industry was established in the United States toward the end of the last century and since its beginning has played a vital role in the production of animal products for human consumption. Today it ranks among the top 15 manufacturing industries in this country, producing approximately 40 million tons of feed annually, with a value in excess of three billion dollars. Census of manufacturers data for 1957 lists 2,016 companies employing 38,010 production workers. According to trade estimates, these figures account for only one-half of the feed manufacturing firms in operation. Since 1950 the decline in the number of farms coupled with increased competition has resulted in fewer new firms entering the industry. During the early developmental period the feed mills manufactured a limited number of complete feeds ready for feeding to the animal, and a hand scoop shovel was the basic mixing tool of the feed mill worker. Today many mills offer over 300 different feed formulations. These include complete feeds, premixes, and an increasing number of concentrates for mixing with the farmer's grain. The trend in the industry is toward decentralization with smaller automated plants located near the consumer and producing a wide variety of feed products. (1)

Concurrent with the production of concentrates, increasing interest was manifested in the health status of the feed mill workers. A number of the ingredients in the concentrates are recognized skin, or respiratory, irritants or sensitizers. Among these are flaxseed, soybean meal, penicillin and other antibiotics, sulfa drugs and organic arsenicals. (2)

In addition, diethylstilbestrol (DES), a cattle-feed supplement, had been reported to produce feminizing effects in some pharmaceutical workers exposed to the pure compound<sup>(3)</sup> and is considered by some authorities to be a potential carcinogenic agent in man.<sup>(4)</sup> A further concern was that permanent respiratory tract damage might occur following excessive exposure to the various grain dusts and fungi present in the feed mill environment.

In order to identify the health hazards associated with the various feed mill operations and, at the same time, define the health status of the feed mill worker, a survey of the formula feeds industry was proposed in 1960. Preliminary to the study, contacts with management and trade union officials failed to disclose a significant health problem in the mills. With the exception of one British report describing a study of fungi in an animal provender mill,<sup>(5)</sup> no information regarding the health aspects of the formula feeds industry was found in the literature. Since potential occupational hazards were known to be present in the formula feed mills and no information was available upon which to base corrective measures, if necessary, a comprehensive environmental and medical study of three midwestern formula feed mills was conducted by personnel of the Division of Occupational Health of the Public Health Service. Mills of different basic types in other sections of the country were also visited in order to obtain some comparative information.

A field team was composed of two physicians, three industrial hygienists and one x-ray technician. The physicians were responsible for

the following procedures; medical history, occupational history, physical examination, pulmonary function test and urine collection. The industrial hygienists were responsible for the environmental studies. A chest roentgenogram of each worker participating in the study was taken and developed by the technician.

Estrogen bioassays were contracted to a private organization, The Endocrine Laboratories of Madison, Wisconsin. Chemical DES analyses and metal analyses were done by personnel of the Physical and Chemical Analysis Section, Research and Technical Services Branch, Division of Occupational Health.

This report covers a description of the study, the environmental and medical findings, and recommendations for the control of health hazards in the feed mill environment.

## ENVIRONMENTAL STUDIES

### Description of Operations

Wide variation in process, operation and plant layout is an inherent feature of the animal feed mill industry.<sup>(1)</sup> In all except the recently built mills, equipment and physical plant growth give evidence of an evolution from the old grain mill and feed store. Figure 1 is a schematic flow diagram for a composite of the majority of mills now in operation. It depicts both the "line" and "batch" processes. This combination of processes would seldom be found in a single plant: the newer mills ordinarily use the automatic batch method.

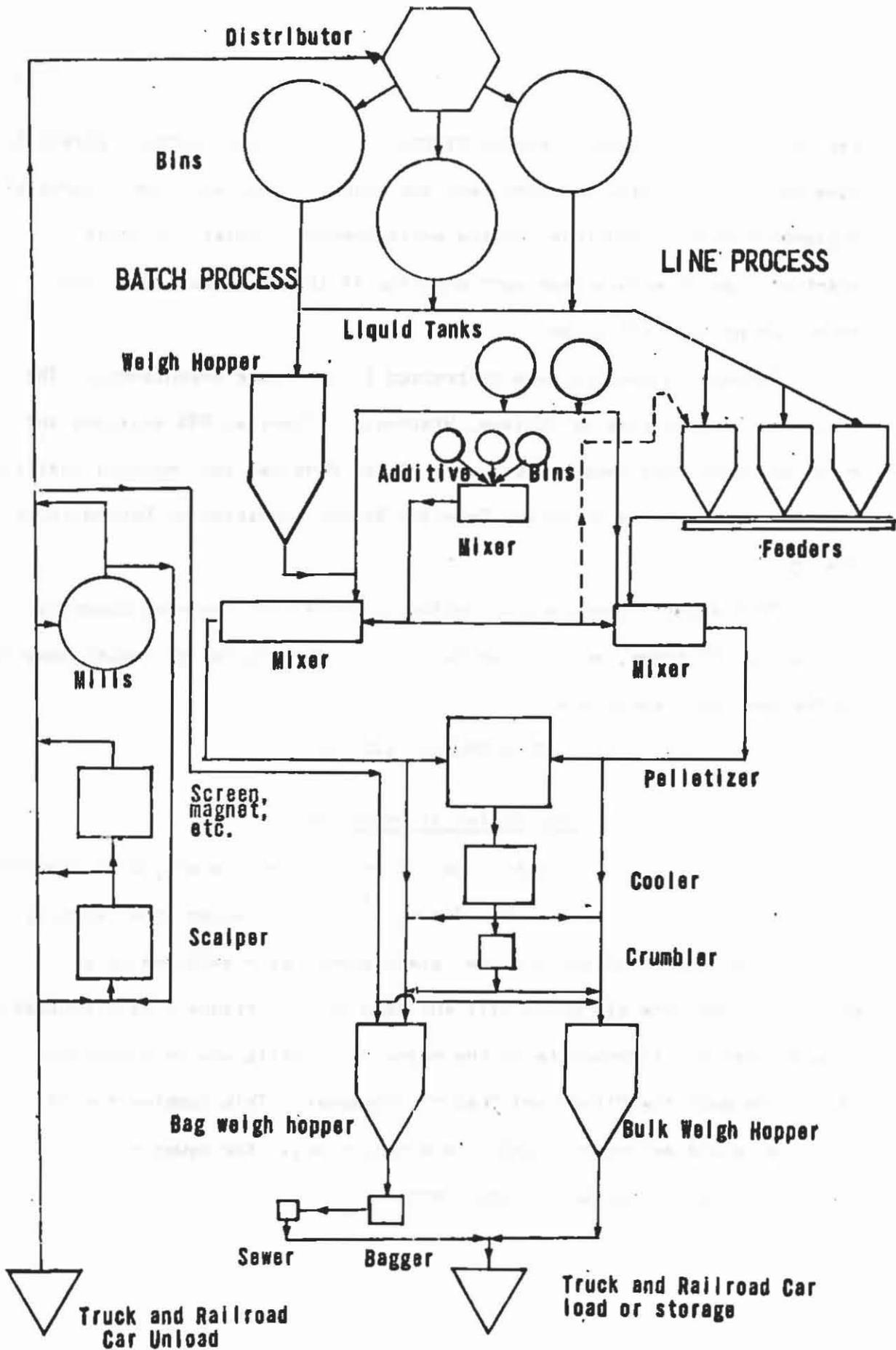


Figure 1

Schematic Flow Diagram

Trucks and boxcars are unloaded into a receiving hopper with power shovels, payloaders or pneumatic devices. Screw, bucket or pneumatic conveyors transport the raw materials to: scalping and separating machines (some are electrostatic or magnetic), dryers, grinding mills (hammer, centrifugal impact, roller, attrition, etc.), or other particle reduction machines, bin distributor system or in some cases, directly to the weigh hoppers. Depending upon the type of raw material, any one or all of these steps may be taken, and it is for this reason that if automation is utilized to any degree, it is put in at this stage of the operation. Raw materials remain in storage bins until they are needed for a run. The type of process used from this point on can now be termed batch, line, or infrequently, both. In the batch process the constituents of a given feed, with the exception of the additives, are weighed in a large hopper which empties into a large mixer. After the pre-mixed additives (usually in a carrier) and the liquids (molasses, fish oils, animal fat, water, etc.) are added, the mixer forms several tons of homogeneous feed mix. The line process differs in that each feed constituent is put in a bin which supplies a single automatic feeder which is set to deliver the proper percentage of material for a given feed mixture. A line of these feeders continuously delivers all of the feed components to a belt conveyor which loads the mixer. This mixer can be smaller than that used in the batch process because it operates continuously with the feeders. Additives are handled, as in the batch process, or they may be put in one of the feeders. The batch process generally involves less worker contact with feed ingredients in the modern mills.

The material leaving the mixers may be pelletized, then cooled and sized. It is sometimes disintegrated in a pellet crumbler, or it can frequently be sent directly to the final weigh hoppers as a mash. The material may then be bagged or put directly into some type of tote-bin. The finished product can then be put in trucks or railroad cars by a variety of materials handling methods. It should be obvious, from even the simplified flow chart shown in Figure 1, that well engineered materials handling methods are the key to an efficient feed mill. The degree of environmental contamination is primarily determined by this factor. The basic characteristics in the three surveyed mills (henceforth identified as A, B, and C) are summarized in Table I.

#### Sampling Methods

A comprehensive environmental study of an industry such as the formula feeds industry requires that an appreciable number of samples be taken by a variety of methods. Air sampling must include determination of gases and solid materials of widely varying particulate size. The amount of air which must be sampled is governed by the concentration of the contaminant in the air and the sensitivity of the analytical method to be applied. Since these factors were, for the most part, unknown in this industry, a certain amount of experimental sampling was undertaken.

#### Filter Samples

High volume samples were taken with Hurricane Air Samplers (Model 16000)\* mounted on tripods at breathing zone levels. Portable battery operated Gast pumps (Model AD440)\*\* were used when it was

\* Gelman Instrument Co., Chelsea, Mich.

\*\* Gast Mfg. Corp., Benton Harbor, Mich.

Table I

Basic Characteristics of Three Midwestern Mills

Plant	Capacity (tons/day)	Approximate Number of Production workers	Type of Process	Method of Handling Additives
A	150	33	Batch	DES and arsenicals are dumped, by sackfulls, into floor hoppers. Other additives, including all minerals, are made up at a secondary plant where workers scoop materials into a scale. Some mixing is done on the floor near the mixer hopper opening. Premix is then taken to appropriate floor hoppers for hand dumping.
B	100	25	Batch	All additives are proportioned by weighing on a small scale and mixed in a room separate from the rest of the plant. A carrier is utilized. Premix is dumped into floor mixer hoppers.
C	200	81	Line	Many additives (including most of minerals) are purchased in a mixed form and are dumped into draver feeder hoppers on main floor level. DES and arsenicals are likewise dumped, by bagfulls, into other draver hoppers. The feeders are located at a basement level and continuously proportion material onto a mixer feeder belt.

desired to follow a workman and obtain samples at his breathing zone level. Table II outlines the use of the filter samplers. It should be noted that the use of polystyrene fiber filters and high volume samplers facilitated the procurement of large amounts of airborne dust in a short length of time (300 - 500 mgm in 1/2 hour). This is of prime importance when sampling for trace amounts of additives such as DES.

Workroom air samples were taken at stationary positions; battery operated samplers were used near workers who were moving about the plant. Since the filter samples were obtained at a known flow rate for a specific time, analysis of the collected material permitted determination of the aerosol concentration.

#### Settled Dust

Airborne dust which had settled out on rafters and other relatively high projections was analyzed for most of the feed materials which were of interest in this survey. Since large, non-respirable, dust particles, which have a relatively rapid settling rate, are removed from the air before they can be deposited on high surfaces, this type of sampling can give a fair estimate of the composition of airborne particulates.

#### Impinger Samples

Airborne dust was drawn into midget impingers which contained water. The captured dust particles were then counted under a light field microscope using standard techniques. (6) The relative "dustiness" of different mills and of feed mills as compared to other industries was thus obtained.

Table II

Air Sampling Method

<u>Instrument</u>	<u>Filter Media</u>	<u>Sampling Rate</u>	<u>Feed Material</u>
Hurricane 4" head	Polystyrene Filters Microsorban*	15-20 cfm	Minerals, arsenous compounds and DES
"	Membrane Filters AM 4*	5-7 cfm	Minerals and arsen- ous compounds
Gast 2" head	"	0.2-0.3 cfm	Minerals, arsenous compounds and my- cologic samples
"	"	" for very short time	Dust size analysis (6)

\* Gelman Instrument Co., Chelsea, Mich.

### Carbon Monoxide

The Bacharach\* "Monoxor" field sampling equipment was used in those areas where it was felt that an exposure to carbon monoxide was possible. This type of instrument gives a direct reading indication of the presence of carbon monoxide in the physiologically significant levels.

### Mycologic Samples

The mycological investigation was necessarily designed to determine the presence of as many organisms as possible since so little is known about the specific mycologic population of grain and processed grain dust. Three types of samples were collected for analysis: (a) airborne dust samples collected in midget impingers filled with sterile, normal saline solution, (b) airborne dust samples collected on the surface of one inch membrane filters, and (c) "grab" samples of feed materials taken from storage bins and boxcars. More restrictive sampling and analytical methods would be required for further identification and quantitation. All of the samples were diluted and plated out on various types of media which are formulated to restrict bacterial growth and enhance the growth of certain types of fungi. A description of the analytical technique will be found in Appendix III.

### Results of Environmental Studies

#### Diethylstilbestrol (DES)

DES is obtained at most mills in the form of a concentrate utilizing soybean oil flakes as a carrier (one gram of DES per pound of concentrate). This concentrate is added to the other feed materials

\* Bacharach Co., Pittsburgh 8, Pa.

to make an 0.0011% or a 0.0022% mixture. High volume aerosol samples were obtained at all locations where atmospheric contamination with DES appeared likely. Several similar samples were taken at points distant from these locations as well. Analytical methods for DES determinations are outlined in Appendix I.

Aerosol levels of DES are shown in Table III. DES was found in all samples when it was being used in the plant, regardless of the location of sampling equipment. It is not possible to find significant differences in concentration which could be attributed to any noticeable environmental parameter. Furthermore, no difference in airborne levels of DES between operations involving 0.0011% vs. 0.0022% feed mixtures was observed. A number of special DES samples which separated the "respirable" from the "non-respirable" particulates were taken following the mill survey. Using a cyclone-type separator,<sup>(7)</sup> designed to classify particles above and below the respirable size limitation point (5 microns in diameter, unit density 1.0) it was found that up to 60% of the DES concentrate aerosol was in the suggested respirable range.

No threshold limit values (TLV) have been determined for DES\*. No published work on the toxicology of airborne DES has been found by the authors. Watrous and Olsen<sup>(3)</sup> have stated that clinical evidence of exposure is seen after urinary excretion exceeds 0.10 microgram per millimeter. They further postulate, using several acknowledged assumptions, that a man must absorb 0.30 to 1.0 milligrams of DES per day to show this level in the urine. These values were obtained from a relatively small

\* A threshold limit value is the atmospheric concentration of a material to which workers may be exposed for an eight-hour working day, day after day, without injury to health being expected.

Table III  
DES Analyses of Aerosol Samples

High volume samplers, operating for long periods of time, were used in obtaining these samples. A complete work cycle (mixing, bagging, etc.) was sampled when possible.

Plant	Location	Feed	Concentration DES ( $\mu\text{g}/\text{m}^3$ )*
A1	Bagger	Beef feed (0.0022% "DES")	0.16
"	Bagger	Beef feed (0.0011% "DES")	0.11
"	Bagger	"	0.45
"	Hopper	"	1.03
"	Hopper	"	0.74
"	Sewer	"	0.02
"	Hopper	Beef feed (no "DES-A" "clean-up" operation)	0.02
"	Bagger	"	0.03
B	Pre-Mix	Beef feed (0.0011% "DES")	0.33
"	Bagger	"	0.02
C	Bulk Load #1	Beef feed "A" (0.0011% "DES")	0.13
"	Pelletizer	"	0.04
"	Pelletizer	"	0.07
"	Bagger #1	"	0.04
"	Pelletizer	Beef feed "B" (0.0011% "DES")	0.18
"	Draver Feeder	Baby beef feed (0.0011% "DES")	0.03
"	Draver Hoppers	—	0.06
"	Near Office	—	0.14
"	Near Draver Room Door	Beef feed "A" (0.0011% "DES")	0.03
"	Draver Feeders	Baby beef feed - no "DES" (other feeds with DES 1 hour before)	0.24
"	Draver Feeders	Beef feed "A" (0.0011% "DES")	0.74
"	"	"	0.54
"	"	Beef feed "B" (no "DES")	0.21
"	"	"	0.15
"	Bagger (Custom)	Baby beef feed (0.0011% "DES")	0.02
"	Bagger (Bulk)	"	0.02
"	Bagger (Bulk)	"	0.07
"	Bagger (Custom) (Bulk)	Beef feed "A" (0.0011% "DES")	0.24
"	"	"	0.11
"	Bagger #1	Beef feed "B" (0.0011% "DES")	0.08
"	General Bag & Mix Area	—	0.20

\* micrograms per cubic meter of air

number of men over a short exposure period, but it is therefore estimated that from 30 to 100 micrograms of DES per cubic meter of air must be inhaled during the course of an 8 hour day in order to reach the clinical manifestation stage. This level is 30 to 100 times higher than the highest airborne concentration found in any of the mills at the time of the environmental survey. The values in Table III appear to be quite low on the basis of present toxicity information on DES.

It is quite obvious that the opportunity for skin absorption and ingestion is abundantly present in the mills. Workers were observed smoking, eating and moistening fingers with the tongue during work periods. Work clothing becomes impregnated with feed materials, since one clothing change a week during the winter months is the practice. Special efforts to avoid handling the DES concentrate are not generally made. There is little doubt that low level exposures do occur via the inhalation, skin absorption and ingestion routes. These exposures are not daily, nor are they likely to continue over an eight hour period.

Settled dust (rafter) sample results are shown in Table IV. The average concentration was 1.5 micrograms of DES per gram of dust. Sample location seemed to make very little difference in the concentration of DES in settled dust. For instance, both the maximum and minimum values listed above are from samples taken in the same mill at locations which are rather close to one another. This is consistent with the detection

Table IV

DES Analyses of Settled Dust

Samples of air-borne dust which had settled on rafters and other projections.

Plant	Location	Concentration DES ( $\mu\text{g/g.}$ )*
A	Truck drive	0.68
A <sub>1</sub>	Mixer "E"	0.68
"	Hopper "B"	1.91
"	Bagger "A"	4.10
"	Hopper "A"	1.01
B	Hopper	5.05
"	Pre-Mix	0.70
"	Bagger	0.35
C	DES draver hopper (near bins)	0.91
"	DES draver hopper (lower level)	1.20
"	Bagger	1.64
"	Wenger Line (near ceiling)	0.64
"	Near office	1.20
"	Roof rafters	0.96
"	Storage area	1.37
"	Room opposite DES hopper	1.93
"	Mineral hopper	1.65
"	Bagger #1	1.04

\* micrograms per gram of dust

of airborne DES at a point more than 100 feet from the nearest generating source. A comparison of Tables IV and VII (settled mineral dusts) would tend to indicate that the DES concentrate particles are at least as capable of becoming airborne as are the mineral additives.

#### Organic Arsenic

A number of air samples were analyzed by the molybdenum blue method for arsenic.<sup>(8)</sup> Results are shown in Table V. All samples were well below the TLV of 500 micrograms of inorganic arsenic per cubic meter of air.<sup>(9)</sup> However, toxicologists have suggested that one-tenth of this value be used as the limit for exposure to organic arsenic compounds.<sup>(10)</sup> Two samples at bagging stations were above this level, demonstrating that it can on occasion be exceeded. Improved handling methods should eliminate these occasional high exposures.

#### Trace Mineral

A quantitative spectrographic method was employed for the determination of manganese, copper, zinc, iron and cobalt in air and settled dust samples (see Appendix II). Atmospheric concentrations of these metals are believed to represent a reasonable index of the atmospheric concentrations of other minerals which are added to the feed mixture. With only a few exceptions, all aerosol samples for minerals showed concentrations of these metals well below any established or suggested TLV.<sup>(9, 10)</sup> Table VI lists the results of analyses of mineral samples.

The charging of mixer hoppers seems to be the operation which results in the higher concentrations. However, only a few of the

Table V

Arsenic Analyses of Aerosol Samples

These samples were obtained with a high volume filter sampler at "breathing zone" levels.

Plant	Location	Feed	Concentration As ( $\mu\text{g}/\text{m}^3$ )*
A <sub>1</sub>	Hopper "A"	35% Pig concentrate	24.2
"	Hopper "A"	"	12.7
"	Bagger "A"	"	74.3
"	Bagger "A"	"	16.0
"	Bagger "B"	Hog supplement (no arsenic)	0.2
"	Bagger "B"	Pig starter	2.3
"	Bagger "B"	"	2.7
"	Bagger "A"	"	10.7
"	Hopper "A"	Turkey feed	52.7
B	Hopper	Hog supplement	0.6
"	Bagger	"	1.3
"	Bagger	"	11.8
"	Hopper	"	25.0
C	Bagger (custom)	Turkey conc. feed	2.2
"	Bagger #1	Medicated pig. conc.	1.1
"	Draver Feeder	Hog growth stimulant	1.1
"	Bagger #1	"	3.0
Suggested limit for Organic Arsenic Compounds <sup>(10)</sup>			50.0

\* micrograms per cubic meter of air

Table VI

Mineral Analysis of Aerosol Samples

These "breathing zone" samples were collected at work stations and in some cases taken near a worker who was moving from place to place. Both high and low volume filter samples were used.

Plant	Location	Feed	Mn	Concentration ( $\mu\text{g}/\text{m}^3$ )**			
				Cu	Zn	Fe	Co
A <sub>2</sub>	Bagger	Mineral mix "A"	13	13	*	40	7
"	Scale	"	50	6	52	83	3
"	Scale	Mineral mix "B"	139	13	559	376	96
"	Hopper	"	731	14	856	39	83
"	Bagger	"	10400	2510	6200	30900	11400
"	Sewer	"	1600	283	274	2710	480
"	Sewer	Mineral mix "C"	362	12	22	580	28
"	Bagger	"	32	10	*	119	3
"	Hopper	Turkey feed	213	13	104	34	20
"	Hopper	"	26	9	*	81	5
A <sub>1</sub>	Bagger "A"	32% Beef feed	20	5	23	46	3
"	Bagger "A"	32% Beef feed (0.0011% S)	14	30	34	29	1
"	Sewer "A"	32% Beef feed	9	3	8	31	2
"	Near hopper "A"	Several feeds (mixed)	11	29	219	48	5
"	Hopper	Beef feed	19	2	13	50	2
"	Bagger "B"	Hog supplement	8	5	52	233	<1
"	Bagger "B"	Hog supplement	18	10	80	216	4
"	Hopper "B"	Calf feed	17	2	11	253	3
"	Bagger "B"	Pig starter	5	12	136	52	4
"	Bagger "B"	Pig concentrate	12	9	*	74	2
"	Hopper "B"	Poultry mixing concentrate	193	67	307	211	8
"	Hopper "B"	Brooder concentrate	32	17	261	100	7
"	Bagger "B"	Brooder concentrate	42	19	38	59	2
"	Bagger "B"	Hog concentrate	39	9	*	162	3
"	Bagger "B"	Sow concentrate	16	4	29	95	1
B	Pre-Mix	Pig & lamb concentrate	12	3360	49	90	<1
"	"	Pig & lamb concentrate	<1	1140	*	8	<1
"	"	Sow concentrate	7	35	19	14	<1
"	"	Pig & lamb concentrate	11	641	15	84	1
"	"	Poultry concentrate	337	33	38	86	9
"	Hopper	Turkey holding feed	119	28	661	407	14
"	"	Turkey holding feed	7	4	27	132	<1
"	Truck load	Beef feed	7	49	69	49	3
"	"	Beef feed	13	14	122	71	6

cont. Table VI

Plant	Location	Feed	Concentration ( $\mu\text{g}/\text{m}^3$ )				Co
			Mn	Cu	Zn	Fe	
C	Pelletizer	Pig concentrate	15	39	*	36	3
"	Loading dock	Pig concentrate	124	12	*	133	3
"	Bagger #2	Mineral mix	31	4	86	294	<1
"	Draver feeders	Pig concentrate	2	1	39	28	<1
"	"	Dairy concentrate	15	2	19	20	<1
"	"	Medicated pig concentrate	3	1	11	26	<1
"	Draver hoppers	(Several feeds)	5	26	*	12	*
"	Draver feeders	Beef feed	50	17	32	240	13
"	"	Mineral mix	21	5	18	50	3
"	Bagger #1	Pig concentrate	2	<1	16	6	<1
"	"	Baby beef feed	26	6	*	15	1
"	Bagger (custom) (During bagging and mixing)	Zinc oxide pre-mix	—	—	3120	—	—
"	Bagger (custom) (Bagging only)	Zinc oxide pre-mix	—	—	25400	—	—
"	Bagger (custom) (During bagging and mixing)	Zinc oxide pre-mix	—	—	5520	—	—
Suggested limits for repeated 8 hour exposures <sup>(10)</sup>			600	100	300-1640	10000	100

\* Amounts below sensitivity of method

\*\* micrograms per cubic meter of air

analyses show values which approach a hazardous level, and these occurred in short term operations.

Even though the repeated average daily exposure does not exceed the threshold limit value, some short term exposures to very high concentrations of minerals can cause temporary respiratory discomfort or skin irritation. For example: mineral dust levels found in the premix area of one mill were often higher than the applicable threshold limit values; it was reported, and also observed, that the environment in this plant often causes upper respiratory discomfort. A rudimentary exhaust ventilation system and nuisance dust type respirators are used in an effort to reduce this discomfort. At Mill C, a zinc oxide-dried milk powder premix was prepared; the airborne concentration of zinc during one operation was  $25.5 \text{ mg/m}^3$ . This is well above the suggested limits. This type of exposure is seldom encountered because of the infrequency of production of this product. The workers reported that skin irritation has occurred after handling this material. Workers at Mill C described "uncomfortable" conditions occurring while certain minerals are unloaded from boxcars. During the survey period, a lime car was unloaded. Since this is an unventilated operation, and because the prevailing wind was toward the plant, high airborne dust concentrations were found in the production area of the building.

Spectrographic determinations on settled dusts indicate that the mineral constituents of the feed mixtures become airborne and are distributed about the mill in about the same degree as other feed materials.

Mineral concentrations were approximately the same in these samples as in the feed mixtures. However, much higher concentrations were found in areas where mineral mixes are put in mixer hoppers. The results of analyses for minerals in settled dusts are shown in Table VII.

Particle size analyses were made on a number of air samples. Since it is virtually impossible to identify the various feed constituents microscopically, particle size data can be reported only on the feed dust as a whole. These analyses demonstrate that up to 60%, by count, of the airborne dust particles are of respirable sizes.

Light field microscope dust counts were made on a number of midget impinger samples. These were done primarily to obtain information on relative dustiness between different locations in the mills. The particle counts ranged from 188.70 to 0.25 million particles per cubic foot of air, and the average was 16.78 mppcf. The higher counts were usually found in boxcar unloading or mixer hopper loading operations.

#### Mycologic Samples

Grain dusts are sources of fungal growths of many types. (5, 11) Because certain fungi can cause human disease, gross samples of feed mill dusts were qualitatively and, to some extent, quantitatively analyzed for fungi (see Appendix III). These analyses showed, as was expected, that large quantities of a number of species were present. Among the species present, there were some which have been implicated in certain pathologic conditions. These include: the allergenic types Homodendron and Alternaria; and M. heteromorphum, G. candidum and A. fumigatus. The

Table VII

Mineral Analysis of Settled Dusts

Samples of air-borne dust which had settled on rafters and other projections.

Plant	Location	Concentration $\mu\text{g/g}^{**}$				Co
		Mn	Cu	Zn	Fe	
A <sub>2</sub>	Hopper	16.60	36.80	25.20	41.40	*
" <sup>2</sup>	Scale	5.90	32.40	7.20	11.10	*
"	Pelletizer	0.21	0.43	0.70	5.84	*
A <sub>3</sub>	Bagger	0.08	0.66	0.20	1.36	*
"	Hopper	0.07	0.07	0.20	1.01	*
A <sub>1</sub>	Hopper	0.13	6.92	0.19	3.10	0.006
" <sup>1</sup>	Bagger "B"	0.14	5.50	0.20	3.78	0.004
"	Bagger "A"	0.06	1.40	0.30	4.20	0.005
B	Bagging Room	0.04	0.15	0.29	0.41	*
C	Bagger (custom)	—	—	6.56	0.38	—
"	Wenger line	0.08	0.06	0.51	10.42	*
"	Wenger line (near ceiling)	0.07	3.73	0.48	0.73	0.005
"	Near office	0.27	3.86	0.61	0.45	*
"	Near mineral mix hopper	0.39	0.24	0.59	2.08	*

\* Amounts below sensitivity of method

\*\* micrograms per gram of dust

total population of microorganisms in the samples was so great that precise quantitation of specific species of fungi was not accomplished. Results of the clinical examinations therefore must serve as the best indication of the presence of or absence of mycotic disease.

It should be recognized that under different mill conditions gross exposures to grain dusts bearing a different mycologic population could result in allergic responses or mycotic disease. Feed mills in other areas of the country undoubtedly use grains which show a different mycologic population than the mills in which samples were taken.

#### Grain Dusts

There have been reports of illness apparently resulting from exposure to grain dusts, per se.<sup>(2)</sup> Since these reports do not identify the causative agent, and the environments sampled in this study contained many constituents in addition to grain dusts, atmospheric sampling to specifically explore grain dust exposures was not practical. Workers in each plant mentioned certain dusts which were especially irritating. Although there was some consensus of alfalfa and soybean dust, practically all of the bulk products were mentioned. Reported responses such as temporary skin and upper respiratory tract irritation varied with individuals although no serious or chronic problems were mentioned. Soybean dust was said to contain large quantities of "field dust" at certain times. Chemical analysis showed a very low concentration (2.14 milligrams per cubic meter) of quartz in airborne dust generated during the unloading of a bean shipment.

### Carbon Monoxide

Carbon monoxide (CO) would ordinarily not be a problem in most areas of the mills during the warmer months. In those mills where certain areas of the plant can be closed off during cold weather, the use of industrial trucks (payloaders, fork lift, etc.) with internal combustion engines can result in high CO levels. Certain storage areas of Mill C had CO levels slightly above 50 ppm. If doors and windows are closed during the winter months, CO levels could rise to the TLV of 100 ppm when internal combustion engines are operating.<sup>(9)</sup> Boxcar unloading with industrial trucks is an operation likely to result in high exposure.

Carbon monoxide and carbon dioxide are potential hazards during clean-out operations in any type of large storage tank. Several fatalities ascribed to CO or CO<sub>2</sub> poisoning have been reported in the feed mill industry.<sup>(12)</sup> In these instances the workers were overcome while cleaning out molasses tanks. Some states have codes which specify protection for workers who must clean out such tanks. Since tank cleaning was not undertaken at any of the mills visited during the period of the survey, no investigation of tank exposures was possible.

### General Observations

#### Other Feed Additives

A representative list of feed materials appears in Appendix IV. There are many feed additives, other than those discussed in this report, which have potential health problems associated with their use. The relatively well known allergic reactions to antibiotics is a good example. No attempt was made, beyond observation of work habits, to evaluate exposures to

additives of this type. No gross over exposures to these materials were observed. When such a large number of potentially hazardous materials are used in a single environment, and when few of the materials have an established TLV, the primary means of detection of occupational disease must be based upon clinical evidence.

#### Hygienic Practices

Throughout the course of this study, careful attention was given to the observation of work habits which might have bearing on exposure to potentially toxic materials by ingestion and skin contact. In each of the mills, some degree of carelessness was observed in both types of exposure.

The opportunity for ingestion occurs with practices such as: moistening fingers with tongue (to obtain a firmer grasp on feed sacks or labels), eating food before washing the hands, smoking on the job (also an explosion hazard) and biting the fingernails. These practices were seen in all phases of feed mill operation, including the handling of the more hazardous premix additives. Skin contact was even more prevalent. There seemed to be no conscious effort to refrain from handling any of the feed materials. Workers often become covered with feed dust, and although showers are available, they are seldom used before leaving work. Feed mill dusts, as a general rule, do not cause obvious soiling of clothing; work uniforms, therefore, are often worn for many days before laundering at home. Approximately one-half of the workers are in the habit of changing clothes before leaving work. These work habits can obviously lead to prolonged and, in some cases, excessive skin exposures. In many cases the elimination of the chance for exposure would have been a simple matter.

Materials Handling and Ventilation Methods

In many instances, it was observed that with simple changes in handling methods, more comfortable working conditions could be attained. There were numerous points where feed materials were allowed a free fall of several feet, where they were mixed in an open container or on the floor, where they were transferred by shoveling, or where there were openings in covered transfer chutes and ducts through which material could spill or become airborne. Unnecessary exposures to dusts occurred at these locations. Loss of product also occurred here, and frequently the installation of a simple local exhaust system would be of benefit from both a hygienic and economic standpoint. Even the judicious positioning of large man-cooler type fans could be used as a second choice in removing some dust from the mill atmosphere.

It is often possible to change materials handling methods in a manner such that less of the feed materials will become airborne; sacks can be opened nearer the hopper openings and emptied with greater care, chute openings could be positioned closer to the containing space, greater precaution might be exercised in the operation of payloaders, and routine maintenance procedures on the mixers and other grain handling machinery could lead to reducing the amount of air contamination by an appreciable amount. It is apparent that much improvement could be made at the expenditure of relatively little cost or time.

### Feed Mills In Other Areas of the Country

In an effort to determine if the midwestern mills surveyed are typical of the industry, a number of mills of various sizes in other sections of the country were visited. These mills showed a wide variation in age of the plant, number of workers, and types of processes and products. The most obvious conclusion to be drawn from these visits is that there is no typical feed mill. The following are general observations regarding the mills visited:

1. Automation is definitely moving into this industry. New mills usually operate with fewer than one fourth the number of workers required to produce a given amount of feed in a manually operated mill. A mill can be automated to the point where a single control operator and a few maintenance men can produce the same quantity of feed as any of the older, larger mills.
2. Some mills have installed local exhaust ventilation and other control devices which essentially eliminate significant dust exposure. In others there seems to have been no thought given to dust generation or its control and working areas are extremely dusty.
3. There is wide variation among the mills in the manner in which the feed additives are handled. Several mills handled them with extreme caution, comparable to a pharmaceutical firm, while others were extremely careless.
4. Hygienic practices were quite variable; in general, the larger mills tended to be a bit more conscious of this factor.
5. A high incidence of labor turnover was not seen in this industry.

Certain situations seemed to vary with geographical location:

1. The use of most of the additives, quite naturally varied with the type of livestock and grains indigenous to the area. For example, southern Florida produces little corn or other "beef grains" therefore most of the cattle are fed on open range. Beef feeds in general, and especially those containing DES, are low production items in this area.
2. Purchasers in certain areas of the country have been slow to accept feeds containing the new additives.
3. Portable mixers operate primarily in the midwest where the farmers have abundant supplies of home grown grain.
4. In the warmer areas of the country, mill ventilation is less difficult to achieve because comfort heating is not a major problem.

The general trend in the use of additives seems to have stabilized. Old medications are constantly being replaced by new, but the total quantity of additives has not appreciably changed in recent years. Feed mill managers find that the competition is keen, and the degree of automation and availability of favorable transportation appear to be the primary factors for success.

Although each of the mills visited was unique, it is felt that the intensive study made in the midwestern mills has yielded information which is reasonably representative of the health status of workers in the industry, and that the conclusions and recommendations expressed in this report may be applied to the industry as a whole.

### MEDICAL STUDIES

The medical studies included a medical history, physical examination, chest roentgenogram, pulmonary function test and various laboratory analyses.

#### History and Physical Examination

The medical history was taken to obtain information concerning the past and present health of the employee. Of specific concern were illnesses which might influence the metabolism of the chemicals of interest in the study, or affect the physical or radiographic findings. The occupational history provided the following information on the past and present employment of each worker: location; duration of employment; work description; and hazardous environmental exposures. In addition, information was obtained regarding potential exposures to formula feeds on the farm from those employees who engaged in part-time farming activities. Physical examination included inspection of the skin; auscultation of the chest; palpation of the abdomen; inspection of the genitalia; and the recording of blood pressure.

#### Pulmonary Function Test

A single-breath test was used to obtain a measure of the ventilatory capacity of the workers. The Wright Peak Flow Meter, a device designed to record the maximum forced expiratory flow rate in liters per minute, or "peak flow", following a single voluntary effort, was selected for this purpose.<sup>(13)</sup> This procedure is convenient and practical for screening a large number of subjects. After preliminary instructions on

the purpose and method of the test and a demonstration by the physician, each subject was required to make three efforts. Only the maximum response is used in making group comparisons.

#### Chest Roentgenogram

A 14 by 17 inch chest roentgenogram of each employee participating in the study was taken and developed in a mobile x-ray unit. A target distance of 72 inches at 200 milliamperes was used.

The films were initially interpreted in the field by one of the team physicians. Workers with conditions requiring immediate medical attention were promptly referred to their physicians. Workers with normal films were not contacted.

Upon completion of the field work the films were sent through a panel consisting of three experienced radiological consultants. If the panel so indicated, letters were sent to the worker and his physician notifying them of a condition requiring further evaluation.

#### The Laboratory Examination

The laboratory examination consisted of bioassay and chemical determinations of diethylstilbestrol (DES) urinary excretion, spectrographic studies of urinary metals, and routine urine tests for sugar, protein and pH.

Urine was selected as the biologic sample for the following reasons: ease of collection, ready acceptance of this procedure by the workers, and available estrogen bioassay methods. A first-morning specimen collected at home was desirable in order to obtain a sample free of

contamination, relatively uniform in concentration and sufficient in volume for the prescribed testing procedures. While a twenty-four hour specimen would have been preferred, conditions existing in the field precluded the use of this technique.

#### Estrogen Bioassay

An extensive estrogen bioassay program was arranged in order to determine whether or not there was evidence of excessive absorption of DES by employees who handled concentrates or feeds containing this estrogen. Three urine specimens from each employee were submitted for bioassay. Since the possibility existed that a variation in estrogen excretion might occur following a week-end without DES exposure, a schedule of collection was arranged to provide two specimens on the fourth working day of the week and one specimen on the first working day. Those men who neglected to collect a specimen as directed were asked to submit one on the following day. A spot sample of urine collected at work was taken in a few instances when the above procedure was not possible.

The specimens were shipped in plastic containers without preservative; however, samples were frozen within 12 hours of collection and maintained in this state until assayed. Since DES is reported to be stable in the urine, no significant loss of activity was expected.

A detailed description of the bioassay procedures including statistical calculations is contained in Appendix V.

In order to establish an internal control for the bioassay procedures, 24 urine specimens from the production and office groups were

selected at random and divided into two equal parts. Known quantities of DES ranging from 0.10 to 0.48 micrograms per milliliter were added to 12 of the resultant 48 specimens, and all specimens were then submitted for testing.

#### Diethylstilbestrol Chemical Analysis

Twelve urine specimens from the third sampling period were selected at random for chemical DES determination (see Appendix I). The test is specific for DES and, consequently, is not affected by endogenous estrogen and androgen as in the bioassay.

#### Urine Metal Analysis

Several of the animal feed supplements are mineral-containing compounds used primarily to increase growth and maintain good nutrition. The following metals were selected as possible indices of exposure for urinary spectrographic analysis: arsenic, cobalt, copper, manganese, and zinc (see Appendix II). Only urine specimens from workers believed to have maximum exposures to one or more of these metals, as determined from the occupational history and environmental data, were analyzed.

#### Routine Urine Tests

A "dip-and-read" test was used for the simultaneous, colorimetric determination of proteinuria, glycosuria and urinary pH.<sup>(14)</sup>

#### Results of the Medical Examination

Approximately 141 production workers were employed by the three mills at the time of the survey. Of this group, 116 men, or about 82 percent, were examined; the percentages in the individual mills were

97, 88 and 74. Twenty-three office workers with little (less than one hour per week) or no exposure in the mills were selected as a control group from two of the mills. This group was not selected at random; in one mill essentially all office workers were included while in the other the employer had previously selected several office employees of income and age comparable to his production group.

Reasons given for non-participation in the study, in order of frequency, were: recent examination, absence due to illness or vacation, refusal with no reason given and religious influences.

#### General Characteristics of the Workers

Sex, Race, and Age: The population studied was 100% white male. Women were not employed in the production capacity by the mills and were not included in the control group. The age distribution of the employee population which is presented in Table VIII shows that both the production and control groups are relatively young. The age pattern of the production workers is similar to that observed in Public Health Service studies of diatomite workers and Utah nonferrous metal miners. (15,16)

Smoking Habits: Smoking was prevalent (83%) in the production group and less frequent (39%) in the control group. The smoking data are presented in Table IX.

#### Duration of Employment

The duration of employment data, presented in Table X, reveal that 78 percent of the production group had worked less than 10 years and 44 percent less than 5 years in the formula feeds industry. For comparison, the proportion of workers with less than 10 years of

Table VIII

Age Distribution of 116 Feed Mill Production Workers and 23 Office Workers

<u>Age in years</u>	<u>Number of production workers</u>	<u>Percent of production workers</u>	<u>Number of office workers</u>	<u>Percent of office workers</u>
Under 20	0	0.0	1	4.3
20-24	18	15.5	6	26.1
25-29	19	16.4	5	21.8
30-34	26	22.4	2	8.7
35-39	18	15.5	4	17.4
40-44	12	10.4	4	17.4
45-49	8	6.9	1	4.3
50-54	4	3.4	0	0.0
55-59	6	5.2	0	0.0
60-64	3	2.6	0	0.0
65 or over	2	1.7	0	0.0
Total	116	100.0	23	100.0

Table IX

Smoking Habits of 116 Feed Mill Production Workers  
(first number) and 23 Control Workers (second number)

Tobacco Usage	No Smoking	Less than 5 years	5-9 years	10-14 years	15-19 years	20-24 years	25 years or more
No use of tobacco	13/9						
Discontinued regular smoker*	7/5						
Cigarette smoker less than 1/2 pack daily		1/0	1/0	0/2	0/1		
Cigarette smoker 1/2 to 1 pack daily		3/2	13/1	15/0	6/0	10/1	11/0
Cigarette smoker more than 1 pack daily		1/0	3/1	3/0	11/0	1/0	7/0
Cigarette smoker 1/2 to 1 pack daily and cigar smoker				1/0	0/1		
Cigarette smoker more than 1 pack daily and cigar smoker				1/0			
Cigarette smoker 1/2 to 1 pack daily and pipe smoker			1/0	2/0			1/0
Cigarette smoker less than 1/2 pack daily and cigar and pipe smoker							1/0
Cigarette smoker 1/2 to 1 pack daily and cigar and pipe smoker							1/0
Cigar smoker only, less than 5 daily			2/0				

\* have not smoked for one year or longer

Table X

Distribution of 116 Production Workers by Years  
Employed in the Formula Feeds Industry

<u>Years Employed</u>	<u>Number of Workers</u>	<u>Percent of Workers</u>
Under 1	8	6.9
1-4	43	37.1
5-9	39	33.6
10-14	17	14.7
15-19	8	6.9
20 or over	1	0.8
	<hr/>	<hr/>
Total	116	100.0

employment in their respective industries in other Public Health Service studies was as follows: (15-19)

Diatomite Industry	85%
Chromate Industry	59%
Foundry Industry	55%
Nonferrous Metal Mines	54%
Open Hearth Steel Industry	35%

### Occupation

A classification of occupations is given in Table XI. Since job transfer occurred frequently and the proximity of operations led to similar exposures, only the two major groups - production workers and office workers - are compared with respect to medical and laboratory findings.

Few jobs in the mills were dust free. While none of the office workers complained of dusty working conditions, 93 percent of the production group registered this complaint.

### Medical Findings

Skin: There were no definite cases of occupational dermatitis in either the production or the control group at the time of the examination. Three cases of contact dermatitis were observed but no relation to occupation was established. There was the usual incidence of other skin conditions. All workers with dermatitis were examined by a dermatologist, one of the physicians on the field team.

Past history revealed that six workers reported contact dermatitis from various feed ingredients, including flax seed and oat seed. No one reported any difficulty from the antibiotic or sulfa additives

Table XI

Occupational Distribution of 116 Feed Mill Production Workers.

Occupation	Number of workers
Mill hands	53
Industrial truck operators	18
Supervisory personnel	14
Maintenance personnel	10
Truck drivers	8
Draver feeder operators	6
Premix workers	4
Miscellaneous (laboratory workers)	3
	<hr/>
	116

in feed, although six workers had a past history of penicillin allergy and two workers had previously developed urticaria following the ingestion of sulfa.

None of the potential effects of DES on the male breast - enlargement, nodules, tenderness or increased pigmentation - was observed.

Heart and Lungs: Serious respiratory or cardiovascular disease was infrequent among the workers in both the production and office groups. Physical examinations revealed the expected incidence of rales, wheezes, murmurs and arrhythmias. A history of chronic cough from one to thirty years duration was given by 10 percent of the production workers; none of the control group had this complaint. Of the twelve men with chronic cough, six stated that the cough had begun prior to feed mill employment. No workers complained of cough aggravation by the dust present in the mills. Further, those workers with a chronic cough were moderately heavy smokers, the group averaging more than one package of cigarettes a day for 16 years, and cough aggravation from smoking was frequently reported. This amount of smoking was uncommon in the control group as previously noted (Table IX) and may explain the observed difference in respiratory complaints between the two groups. Boucot, et al, reporting on the prevalence of cough among older male smokers, found that cough rates increased with increasing amounts and durations of smoking for all smoking categories, and that "cigarette cough" was, indeed, a clinical entity. (20)

The past history did not reveal an unusual incidence of acute upper respiratory infections; however, approximately 20 percent of both the production and control groups complained of "colds" of less than two weeks duration at the time of the survey. Cough was the predominant symptom.

Abdomen and Genitalia: No significant differences in incidence of past illness, current complaints or positive physical findings relative to the gastrointestinal and genitourinary systems were observed between the production and control groups.

Neuromuscular Findings: Of the 116 production workers, 21 (18.1%) complained of recurring headaches of either recent origin or several years duration; none of the control group registered this complaint. Three workers related their headaches to feed mill exposures; two men developed headaches following exposure to exhaust fumes generated by fork-lift trucks in closed areas and headaches occurred in the third man during work with the premix ingredients.

Muscle cramps and rheumatism were frequent complaints (14.7% and 12.1%, respectively) of the production workers and were reported infrequently (4.3% and 0.0%) by the controls. These values are not excessively high for an industry requiring moderate physical exertion and year-round exposure to outside temperature and humidity. For comparison, in a Public Health Service study of 2,950 male ship welders, 16.6% complained of rheumatism.<sup>(21)</sup>

Blood Pressure: Table XII summarizes the results of the blood pressure determinations. Measurements were taken with the worker in a sitting position following the medical history and prior to the physical examination. The reported values for the two population groups are similar and show the expected rise with age. These figures compare favorably with those reported by Lasser and Master in a study of almost 80,000 apparently healthy people.<sup>(22)</sup>

Table XII

Mean Systolic and Diastolic Blood Pressures of 116 Feed Mill Workers and 23 Office Workers According to Age

Age in years	<u>Production Workers</u>			<u>Office Workers</u>		
	Number of men	Mean systolic (mm/Hg)	Mean diastolic (mm/Hg)	Number of men	Mean systolic (mm/Hg)	Mean diastolic (mm/Hg)
Under 20	0	—	—	1	155	100
20-29	37	129	79	11	131	79
30-39	44	133	84	6	128	77
40-49	20	135	91	5	136	87
50-59	10	146	92	0	—	—
60 or over	5	160	90	0	—	—
All ages	116	134	83	23	132	81

Pulmonary Function Test: The results of the pulmonary function test using the Wright Peak Flow Meter to measure the maximum expiratory flow rate are presented in Table XIII. The findings are consistently lower than those reported by Wright in his initial calibration of the instrument<sup>(13)</sup> but are similar to those found by the Public Health Service in a study of silicosis in male metal miners (unpublished data) and by Higgins in a study of 331 British male agricultural workers.<sup>(23)</sup> Duration of time in the mill was not an important factor except as it related to age. Comparison of the production workers to the office workers offers little useful information since the reported mean values of both groups are considered to be normal. Only one worker with a history of chronic respiratory disease was considered to have an abnormal value of 200 liters per minute.

Chest Roentgenogram: A panel of three radiologists reported their interpretations of the chest roentgenograms. In no case was there complete disagreement. No roentgenogram was classified as positive or doubtful in the International Labour Office Classification of Pneumoconiosis. There was a low prevalence of abnormal heart shadows, pleural shadows, old tuberculosis lesions, histoplasmosis and other miscellaneous abnormalities.

#### Results of the Laboratory Examination

##### Estrogen Bioassay

Six hundred and fifty bioassays were reported during the study. Their distribution according to the purpose and method of testing is shown in Table XIV. Sixty-nine percent of the bioassays were urine specimens submitted by the production and office workers, 25 percent were

Table XIII

Mean "Peak Flow" Values of 114 Production Workers  
and 23 Office Workers According to Age

Age in years	Number of production workers	Mean value (liters/minute)	Number of office workers	Mean value (liters/minute)
Under 20	—	—	1	620
20-29	37	514	11	551
30-39	44	505	6	608
40-49	19	468	5	528
50-59	9	419	—	—
60 or over	5	479	—	—
Totals	114	494	23	563

Table XIV

Distribution of 650 Bioassays According to  
Purpose and Method of Test

Bioassay Samples	Oral-Feeding Method of Bioassay	Subcutaneous- Injection Method of Bioassay	Total
Production group - 116 workers	330	48	378
Office group - 23 workers	66	3	69
Laboratory standardization tests	110	54	164
Field standardization tests	27	12	39
	<hr/>	<hr/>	<hr/>
Total	533	117	650

laboratory standardization tests done to establish weekly dose-response criteria and 6 percent were field standardization tests performed as an internal control of the bioassay procedure. All urine specimens were initially tested using an oral-feeding method of bioassay and only those showing significant uterine responses by this method plus a small number of negative specimens were reassayed using a subcutaneous-injection technique (Appendix V). Of the 139 workers examined, 125 submitted the requested 3 urine specimens, 11 submitted 2 urine specimens, and 3 submitted one urine specimen.

Table XV lists 18 men with urine specimens showing highly significant ( $P$  less than 0.01) uterine responses by one or both methods of bioassay. Of these 17 are production workers and one (No. 6) an office worker. No worker had more than one positive urine specimen and 17 of the 18 positive urine specimens were submitted on the fourth or fifth day of the work-week. The corresponding estrogen excretion values as determined from the weekly dose-response curves ranged from 0.020 to 0.056 micrograms per milliliter of urine reported in DES equivalents. Although these values are based upon highly significant uterine responses they fall in relatively flat portions of their respective dose-response curves and, therefore, do not necessarily represent true estrogen excretion values. This can be seen in Appendix VII where known amounts of DES were added to aliquots of urine before assay. Only one urine specimen, No. 10, listed in Table XV, continued to show a highly significant uterine response on reassay by the subcutaneous injection method. In addition,

Table IV

Urine Specimens Showing Highly Significant (P less than 0.01)  
Uterine Responses on Bioassay

Case Number	Age in Years	Job Classification	Duration of employment in years	Time of sample (1)	Type of sample	Oral-feeding Method Week of DES Equivalent assay in micrograms/ml.		Subcutaneous-injection Method Week of DES Equivalent assay in micrograms/ml.		History and physical examination
1	29	Production	9 6/12	3	Spot	3/21/61	0.020	11/2/61	Negative	Negative
2	21	Production	2	3	Spot	3/14/61	0.023	11/2/61	Negative	Atopic history
3	24	Production	2	3	First AM	3/14/61	0.023	11/2/61	Negative	Dermatitis
4	20	Production	1	3	Spot	3/14/61	0.026	11/2/61	Negative	Rheumatic fever history, cardiac murmur
5	34	Production	12	3	First AM	3/14/61	0.029	11/2/61	Negative	Negative
6	36	Office	2	3	First AM	3/2/61	0.029	10/25/61	Negative	Negative
7	65	Production	17	2	First AM	2/20/61	Negative	10/25/61	c.0.03	Diabetes mellitus
8	28	Production	7	3	Spot	3/14/61	0.034	11/2/61	Negative	Negative
9	20	Production	1 7/12	1	First AM	12/5/60	0.035	10/18/61	Negative	Palpable liver
10	38	Production	3	3	Spot	3/14/61	0.037	11/2/61	0.020	Negative
11	23	Production	1 6/12	1	First AM	11/29/60	0.037	No Test	—	Chronic cardiac arrhythmia
12	43	Production	8 6/12	3	First AM	3/2/61	0.037	10/25/61	Negative	Chronic respiratory complaints
13	37	Production	9 3/12	1	First AM	11/29/60	0.038	10/18/61	Negative	Negative
14	51	Production	9 3/12	1	First AM	12/5/60	0.042	10/18/61	Negative	Negative
15	42	Production	10 6/12	1	First AM	12/5/60	0.042	10/18/61	Negative	Excessive alcohol, hypertension
16	37	Production	1 8/12	1	First AM	12/5/60	0.044	10/18/61	Negative	Excessive alcohol, hypertension
17	28	Production	11/12	1	First AM	12/5/60	0.049	10/18/61	Negative	Negative
18	56	Production	3 6/12	1	First AM	12/5/60	0.056	10/18/61	Negative	Palpable liver

(1) Time of sample refers to day of collection. No's 1 and 3 are samples taken on the fourth or fifth day of the work week and No. 2 was taken on the first day.

No. 7 showed activity only by this method. This variability in the bioassay data would seem to preclude a valid interpretation of the estrogen levels reported as being highly significant in the study. A recent unpublished study<sup>(24)</sup>, however, reveals the subcutaneous method to be less sensitive than the oral method for the determination of urinary stilbesterol monoglucuronide, the principle excretion product following low levels of DES absorption. It is not surprising, therefore, that many of the results showing positive responses by the oral method showed negative responses by the subcutaneous method.

The bioassay data furnish suggestive, but not conclusive evidence of DES excretion. The possibility of error in the quantitative interpretation of the results is great at the low levels reported as being significant in the study. They may also reflect the upper limits of normal endogenous estrogen excretion in the adult male. Estrogen values above 0.06 micrograms per milliliter, the range reported by Watrous and Olsen<sup>(3)</sup> to be associated with exposed and imperfectly protected workers, were not observed and would have been detected with reasonable accuracy by either method of bioassay, as shown by the results of the laboratory and field standardization tests (see Appendices VI and VII).

Signs and symptoms of DES intoxication, which are reported to be associated with urinary estrogen excretion levels exceeding 0.10 micrograms per milliliter,<sup>(3)</sup> were not observed in the feed mill workers. Although the bioassay data may seem inconclusive, the negative and low values of urinary estrogen excretion reported in the study support this absence of clinical evidence of DES intoxication.

### Diethylstilbestrol Chemical Analysis

Of the 12 urine specimens randomly selected for fluorometric analysis from the third sampling period, only one was reported to show DES. The level was 5.6 parts per billion. This figure is not too meaningful since it approximates the threshold of sensitivity of the method (5 parts per billion at the time of the test). It is of interest, however, that this urine specimen also gave a highly significant uterine response by the oral-feeding method of bioassay (No. 3 in Table XV), although no uterine activity was elicited when it was reassayed using the subcutaneous injection technique. One other urine specimen (No. 5 in Table XV), showing a highly significant uterine response by the oral-feeding method of bioassay, was reported as negative by chemical determination. The remaining 10 urine specimens were negative by both bioassay and chemical methods of analysis.

### Urine Metal Analyses

The metals of interest in the study - arsenic, cobalt, copper, manganese and zinc - were not present in significant amounts in the urine specimens selected for spectrographic analysis.

### Routine Urine Tests

Four-hundred urine specimens were tested in the field for protein, sugar and pH. Five workers had trace amounts of protein on one or more specimens. The following conditions may account for the proteinuria in four of these workers: hypertension of 2 years

duration; recent history of renal stones, hydronephrosis, and nephrectomy; recent kidney infection; and a current fibrile, respiratory illness. Proteinuria in the fifth worker is unexplained. Seven workers had a positive "dip-and-read" sugar test on one or more urine specimens. Upon retesting these specimens only two workers, one a known diabetic and the other ill with an acute respiratory infection, continued to show positive findings. The pH reactive portion of the test strip revealed all but 5 urine specimens to be on the acid side of the pH scale.

#### CONCLUSIONS

Three midwestern formula feed mills were surveyed for the existence of occupational health problems. Although there were operational differences in the mills visited in other sections of the country it is felt that the midwestern mills which were included in the survey illustrated the potential health hazards of the industry.

Environmental samples and observations of production methods indicated that, during the period of this survey, exposures in excess of specific recommended threshold limits for compounds used as an index of overall environmental exposure did, on occasion, occur. However, due to the intermittent nature of operations and a wide variety of formulations successively processed during the study, the average daily atmospheric concentrations for the compounds used as indices of exposure were generally within suggested limits. There was no doubt though, that the overall dust levels were very high at many operations. The findings give no room for complacency since the compounds selected for study may not have been representative of the hazardous nature of the

environment. These statements are based on the present knowledge of the materials found in animal feeds. In many cases this knowledge is rather limited; this is especially true in the case of low level, long term exposure to most of the additives. Changes in process or in quantity and type of feed additives could result in hazardous exposures. Even with currently used materials and processes, such factors as poor ventilation, poor personal hygiene, and with handling methods which generate airborne dust were observed in various mills and could result in occupational health problems.

Medical examinations were made on 116 feed mill production workers and 23 office workers. The findings were essentially negative. The prevalence of non-occupational disease appeared to be comparable to that reported in medical studies of other industrial populations. Occupational disease was not found in the production group of workers participating in the survey. Chest roentgenograms and pulmonary function tests did not furnish evidence of pneumoconiosis. Occupational dermatitis was not confirmed by physical examination although several workers had reported skin problems following contact with various feed ingredients. Clinical evidence of chemical intoxication was not disclosed by the medical and laboratory examinations. In addition, urine analyses for arsenic, cobalt, diethylstilbestrol (DES), copper, manganese and zinc failed to reveal excessive excretion of these materials.

Although the medical findings with respect to well-defined occupational disease were negative at the time of the survey, caution should be exercised in applying these data to the industry. Selectivity may have had an

influence on the findings since the group examined were, at the time, employed in the industry. Workers who could not satisfactorily adjust to the mill environment may have left the industry. Information was not available either on this group or on those who had retired after working a lifetime in the industry. Many of the potentially hazardous materials in the mill environment have been in use for relatively short periods of time and may have unestablished additive and synergistic effects on the workers. In addition, a large proportion (78%) of the feed mill employees participating in the survey had worked less than 10 years in the formula feeds industry. The effects, if any, of low level exposures might not be evident within this period. Further observation of the workers would be necessary to completely define the importance of this time factor.

#### RECOMMENDATIONS

Based on the environmental and medical studies, observations made throughout the industry, and in accord with good industrial hygiene practices in the handling of toxic materials, the following recommendations are offered:

1. Present materials handling methods should be reviewed by management with the view to developing new or changed procedures which would result in a reduction of the airborne dusts. A specific example of a procedure which might be improved is the operation involving the dumping of sacks of various feed components into mixer hoppers.
2. Good hygienic practices, such as the washing of hands, frequent changing of work clothing, daily showers, and the avoidance of placing contaminated objects (primarily hands) in the mouth will reduce the possibility for ingestion and skin absorption of feed components.

3. Carbon monoxide and carbon dioxide have been suggested as the causative agents in several fatal accidents in feed mills in connection with the clean-out operations in molasses tanks. The operation of payloaders and similar devices with internal combustion engines can cause high concentrations of carbon monoxide in confined spaces.

Excessive concentrations of carbon monoxide from such sources is most likely during cold weather when doors and windows are closed. Adequate ventilation should be provided in these cases.

4. Consideration should be given to the installation of mechanical ventilation systems. The installation of adequate local exhaust systems, for instance, would increase general comfort and might well result in an economic gain through product recovery. The application of local exhaust ventilation would be advantageous in trace mineral compounding, mixer hopper loading, and at certain times during the transfer of grains from boxcars to bins. Good engineering control for other specific sources of potentially toxic dusts should also be considered. Good ventilation practice is described in Industrial Ventilation - A Manual of Recommended Practice (American Conference of Governmental Industrial Hygienists Committee on Industrial Ventilation, P. O. Box 453, Lansing, Michigan).

5. Approved dust respirators can be used to provide protection from intermittent dust exposures. This type of protection should not, however, be considered a substitute for good engineering practices in installation and materials handling methods. A list of approved respirators is provided in Appendix VIII.

6. A program of preplacement and periodic medical examinations should be in effect in the formula feed mills. This program should include chest roentgenograms. Since the trend in the feed mill industry seems to be toward increasing the number and changing the type of additives, feed mill operators should be constantly on the alert for the possibility of toxic exposures of their workers.

APPENDIX I

Analytical Method for Diethylstilbestrol in Urine and Air Samples

The fluorometric method of Goodyear and Jenkinson<sup>(25,26)</sup> was modified to double the possible number of daily analyses, to decrease the error of single determinations, and to reduce the amount of emulsion formation during the extraction stages of the procedure. With the modified conditions, the errors of single determinations were within  $\pm 5.0\%$ , using a working curve for the final estimations of the DES content in the urine aliquots taken for analysis. The sensitivity of the method, as applied to urine samples, with added DES, is 3 parts per billion.

The fluorometric method for the determination of DES in urine samples was modified slightly for its application to dust samples collected on filter media. The increment ratio technique described by Goodyear and Jenkinson was used in the calculations of the DES content of dust sample aliquots taken for analysis. The sensitivity of the method for dust samples is 0.3 microgram of DES. A linear relationship between the varied quantities of DES added as internal standard and the fluorometric readings has been demonstrated during the application of the modified procedure to synthetic samples. Similarly, a linear relationship has been shown to exist between the total concentration of DES in a filter sample, as calculated by the increment ratio technique, and the ratio of the unknown to the known sample's fluorometric readings. The confirmation of these linear relationships attests to the validity of the method.

The following technique was used for the determination of DES in filter and settled dust samples:

1. Cut up the filters and transfer to soxhlet thimbles. Reflux overnight (16 hours) with 200 ml. portions of chloroform.

2. Add a few drops of water\* to the separatory funnels. Remove samples from the soxhlet apparatus and split samples in half (trapped extract around thimble must also be included in the split). Aliquot at this step.

3. Transfer to individual separatory funnels and add one microgram of DES in chloroform solution to one of the half portions of each sample and mix with a rolling motion. The spiked, half portion of each sample will serve as the reference for final calculations.

4. Extract with two 20 ml. portions of 1N NaOH, using globe-shaped, Kimax, 250 ml., short stem separatory funnels and extracting with a rolling type of motion to avoid excessive emulsion formation. A 10-minute settling after each NaOH extraction reduces excessive emulsion throughout the subsequent procedure.

5. Combine the NaOH extracts. Rinse the empty funnel with one 15 ml. portion of chloroform and add to the NaOH extracts.

6. Wash the NaOH extracts with chloroform until the washings are clear. Allow 3 minute settling at this point and after all the following washings and extractions.

7. Transfer to clean beakers and adjust to pH 8 with 4N phosphoric acid, using pH meter.

\*Use double distilled water throughout the procedure.

8. Extract with three 30 ml. portions of chloroform. Discard the emulsion and the pH 8 solution and rinse the funnel with water. Drain separatory funnel thoroughly.

9. Extract with two 20 ml. portions, 1N NaOH. Rinse the empty funnel with water and discard water.

10. This step is a precautionary one designed to remove the last traces of interfering substances. Wash the NaOH extracts with two 15 ml. portions of chloroform and save the emulsion layer. Transfer the emulsion to a 150 ml. beaker and heat on a steam bath to evaporate all chloroform. The NaOH extract in the funnels should be left uncapped to allow the chloroform vapor to escape. Prepare water washed ether. Allow good separation to avoid super saturation of the ether.

11. Add the combined 1N NaOH extracts (which contains the bulk of the DES) to the beakers (from which the chloroform has been evaporated). Rinse the funnels with water and add to beakers. Rinse the dissembled funnels with water and allow to drain.

12. Adjust pH to 9.5 with 4N phosphoric acid (using pH meter). Rinse the electrodes with small amount of water between samples, adding the rinsings to the appropriate sample solutions.

13. Transfer to separatory funnels and extract with three 30 ml. portions of anhydrous ethyl ether (shake gently). Discard the NaOH layer and rinse funnels with water.

14. Wash ether extracts with three 30 ml. portions of D.D. water and discard the washings.

15. Prepare one inch high beds of anhydrous sodium sulfate in sintered glass funnels (Buchner type, 30 ml. capacity, C. porosity). Pour one portion of water washed ether through each of the sodium sulfate beds. Discard these ether washes.

16. Filter the ether extracts, containing the samples, through the ether-washed filter beds, collecting in clean, ether rinsed beakers. Wash the separatory funnels with a little water-washed ether and pour these washings onto the filter beds.

17. Rinse the sodium sulfate beds with a final portion of water-washed ether, collecting portion in the beaker containing the ether solution of the sample. Immediately invert the sintered glass funnels which were used for the sodium sulfate beds and run distilled water through them, washing away the sodium sulfate. Rinse the funnels with plenty of hot water, then cold water, distilled water and finally with D.D. water. Dry in a thoroughly clean oven reserved solely for the glassware used for this procedure.

18. Evaporate samples to dryness on a steam bath.

19. Dissolve the residue and transfer quantitatively with 2 ml., 2 ml., and 1 ml. 3A S.D. alcohol (absolute Methyl alcohol - 5 ml. and dehydrated ethyl alcohol 95 ml. - analytical reagent grade) to quartz tubes, making up to exactly 5 ml. Then mix but avoid contact with the stoppers. Transfer 2.5 ml. of each sample to matched test tubes and cap all tubes with clean black stoppers to avoid evaporation losses. Irradiate the sample portions, contained to avoid evaporation

using an ultraviolet source such as a Hanovia lamp without the filter. For this purpose a support track, mounted on the front housing of the lamp, facilitated the positioning of the test tube rack holding the samples. We allow the lamp to warm up for 5 minutes and then irradiate the samples for 3.5 minutes. Allow the quartz tubes a few minutes to cool and then transfer to matched test tubes.

20. Set the warmed (30 min) fluorometer at 100 by means of riboflavin standard (USPXVB<sub>2</sub>) which contains 0.1 microgram of this substance per ml. This standard is the third of a set of standard riboflavin solutions. A fresh portion is used each day and the working supply is made up fresh every two weeks.

21. Read all samples in the fluorometer, equipped with a primary filter consisting of a combination of Corning Nos. 5850 (CS7-59) and 3391 (CS7-74) to pass 410-420 mu radiation and a secondary filter, Corning No. 3385 (CS3-71), Novial Shade C to transmit above 490 mu. These readings constitute the "before KOH" values.

22. Add 0.05 ml. of 0.1N KOH to each sample and swirl gently. Allow 10 minutes for shifting of the fluorescent spectrum. Adjust the fluorometer with the riboflavin standard and read the fluorescent intensity of the samples. Record readings after exactly 30 seconds exposure in the fluorometer. These constitute the "after KOH values."

23. Calculations: The difference in the readings obtained on a non-irradiated half of a sample after KOH and its irradiated portion after KOH is a measure of the diethylstilbestrol activity.

Table XVI of fluorometric data obtained from a processed sample and the subsequent calculation of the DES content will demonstrate this estimation.

The following method was used for the determination of DES in urine samples:

1. Add a few drops of water\* to separatory funnels to avoid loss through stopcock.

2. Add 100 ml. duplicate portion of each urine sample to individual separatory funnels (refrigerated samples).

3. Add 1 microgram DES in chloroform solution to one of the duplicates.

4. Extract with three, 30 ml. portions of anhydrous ethyl ether. Roll to avoid emulsion formation and allow to settle 15 minutes after each extraction. Combine extracts.

5. Allow three minute settling time for all subsequent extractions and washings. Wash ether extracts with three, 30 ml. portions of water.

6. Evaporate ether extracts, in 150 ml. beakers on a steam bath.

7. Dissolve the residue quantitatively in 6 ml. dehydrated ethyl alcohol, added as three, separate 2.0 ml. portions and transfer these separate portions to a 500 ml. round bottom flask provided with an outer, standard taper joint. Rinse out beaker with three roughly equal portions of a solution of 1.0 ml. concentrated HCl in 100 ml. of water, adding rinsings to the reflux flask.

\*Use double distilled water throughout the procedure

Table XVI

## Sample Calculation of Fluorometric Data

Sample No.	Percent Taken	Aliquot Factor	$\mu\text{g DES}$ Added	Non-Irradiated Before KOH	Non-Irradiated After KOH	Irradiated-Spiked Before KOH	Irradiated-Spiked After KOH	$\mu\text{g DES}$ per sample
1a	25.0	4.0	0.0	20.8	23.0 B <sub>1</sub> )	20.6	39.1(A <sub>1</sub> )	5.2
1b			1.0	20.9	24.7(B <sub>2</sub> )	23.1	65.5(A <sub>2</sub> )	

$$\frac{(A_1 - B_1)}{(A_2 - B_2) - (A_1 - B_1)} \times \mu\text{g DES added} = \mu\text{g DES in aliquot of "unknown" as spike}$$

$$\mu\text{g DES in aliquot} \times 2 \left( \begin{array}{c} \text{half portion of} \\ \text{sample taken as} \\ \text{unknown} \end{array} \right) \times \text{aliquot}$$

$$\text{reciprocal} \left( \begin{array}{c} \text{final solution} \\ \text{aliquot recip-} \\ \text{rocal} \end{array} \right) = \mu\text{g DES in total unknown sample}$$

$$\frac{(39.1 - 23.0)}{(65.5 - 24.7) - (39.1 - 23.0)} = \frac{16.7}{24.7} \left( \begin{array}{c} \text{Ratio of} \\ \text{readings} \end{array} \right)$$

$$0.651 \times 2 \times 4 = 5.2 \mu\text{g DES in unknown sample.}$$

8. Reflux at 105° C for 16 hours or overnight, using a Liebig condenser.

9. Transfer to clean beakers and adjust pH to 8 with 1N NaOH.

10. Extract with three, 30 ml. portions of chloroform and wash the combined extracts with two, 25 ml. portions of 10% sodium carbonate solution whose pH has been adjusted previously to 9.8 with 4N phosphoric acid. Save the sodium carbonate washings and back extract with one 15 ml. portion of chloroform which is added to the original combined 90 ml. chloroform extract. An aliquot portion may be taken at this step.

11. Extract with two, 20 ml. portions of 1N NaOH. Rinse empty funnel with one, 15 ml. portion of chloroform which is then added to the combined NaOH extracts. Rinse empty funnel with water, which is discarded. Wash NaOH extracts with the chloroform already added. Retain the emulsion in the separatory funnel. Wash with another portion of chloroform and again retain the emulsion. Allow the chloroform vapors to escape from the separatory funnels.

12. Transfer emulsions to clean beakers and evaporate to dryness on the steam bath.

13. Add NaOH extracts to emulsion residues. Rinse the funnels three times with water, adding rinsings to the proper beakers. Finally, rinse the separatory funnels, their stoppers and disassembled stopcocks to prevent any trace amounts of alkali from being dissolved by final ether solution of the samples; discard these latter rinsings. Adjust the pH to 9.5 with 4N phosphoric acid.

14. Extract with ether as described in Step 13, wash ether extracts as described in Step 14, and filter through sodium sulfate as described in Steps 15, 16, and 17 of the previously outlined method for filter and dust samples.

15. Evaporate samples for filter and dust samples.

16. Dissolve the residue with several portions of the alcohol solvent (3A S.D.) and making to 10 ml. in volumetric flask. Transfer 5 ml. to quartz tubes. Then proceed in Step 19 of the method for dust samples.

17. Take the two  $2\frac{1}{2}$  ml. sample portions and read as in the filter and dust sample method, but use 1N KOH and allow 15 minutes for development of the fluorescent shift. Take readings after 60 seconds exposure in fluorometer. See Table XVII for sample calculations.

Table XVII

Sample Calculations for DES in Urine Determination

Sample No.	Vol. Urine	Fluorescent Readings				DES Found ppb
		Non-Irradiated		Irradiated-Spiked		
		Before KOH	After KOH	Before KOH	After KOH	
1 <sub>a</sub>	100	15.3	24.3	17.2	30.2	6.4
1 <sub>b</sub>	100	16.7	32.2	18.3	47.3	

$$\frac{(30.2 - 24.3)}{(47.3 - 32.2) - (30.2 - 24.3)} = \frac{5.9}{9.2} ;$$

$$\mu\text{g DES in "a"} = \frac{5.9}{9.2} \times 1.0 \mu\text{g DES ("b" spike)}$$

$$\mu\text{g DES per liter of urine} = 0.64 \times \frac{1000}{100}$$

$$= 6.4$$

APPENDIX II

Spectrographic Method for Heavy Metals in Air Samples

The membrane filter samples are placed in 250 ml. Phillips beakers and 1.0 ml. of Al soln (2.5 mg.Al/ml.) is pipetted into each beaker.

Approximately 2 ml. of C.P. conc.  $\text{HNO}_3$  is added to each sample which is then ashed under watch glasses on a low temperature hotplate ( $130^\circ \text{C}$ ) until the ash remains light colored after 3 or 4 additions of  $\text{HNO}_3$ .

Several drops of  $\text{HNO}_3$  and several ml. of boiling D.D. $\text{H}_2\text{O}$ . are added to the ash. The dissolved ash is then transferred to chemically clean centrifuge tubes along with 3 hot D.D. $\text{H}_2\text{O}$  rinsings of the beaker.

The samples are evaporated to exactly 1.0 ml. in the oven until each sample contains 2.5 mg.Al/ml. in the final volume. If, after spectrographic analysis, the analysis line is too dark, the sample is diluted with an additional quantity of the 2.5 mg.Al/ml. solution in order to keep the Al concentration consistent throughout.

Spectrographic exposure conditions are as follows:

1. Specpure electrodes
2. Waterproofed craters, 5 mm. deep and 4.5 mm. diameter
3. Buffer: 2.5:1, Graphite: LiCl, 5 mg charge
4. Internal Standard: 0.08 microgram In (from  $\text{In}_2\text{O}_3$ )
5. Excitation: 220 Volt D.C. Arc, 9 amps, 5 mm. analytical gap, 20  $\mu$  slit in all positions. Exposure to LiCl burnout

<u>Element</u>	<u>Analysis Line</u>	<u>In Internal Standard Line</u>
Cu	3274.0 A	3039.4
Mn	2576.1	3039.4
Fe	4383.5	3258.6
Zn	3345.0	3258.6
Co	3453.5	3258.6

APPENDIX III

Mycologic Analyses

The following techniques were used for culture and identification:

Liquid impinger samples -

One milliliter of each sample was plated in triplicate in each of three media

Rose bengal-neopeptone-dextrose agar, with aureomycin

Neopeptone-dextrose agar

Sabouraud's glucose agar

One ml of each sample was placed in 9 ml sterile distilled water, mixed by hand shaking, and 1 ml of this dilution plated in each of 3 plates in each of the three different agars.

One ml of the 1:10 dilution was added to 9 ml sterile distilled water and plated as before.

Membrane filter samples -

The filters were cut in eight equal parts. Four of these portions were placed in various dilutions of rose bengal neopeptone-dextrose agar. The fifth section was cleared with Xylene and mounted in lacto-phenol-cotton blue.

Grab samples -

After shaking, a 1:1000 dilution (in sterile distilled water) of the sample media was plated in triplicate or quadruplicate with rose bengal-neopeptone dextrose agar, and 1 ml in neopeptone dextrose agar. Aureomycin was not used in this run. After settling, the supernatant from a 1:100 dilution was also plated in both agars.

APPENDIX IV

Feed Materials

The following materials comprise a representative list of most of the ingredients used in a complete line of animal feeds.

Natural Ingredients

Alfalfa	Feather meal	Milk
Animal fat	Fish meal	Molasses
Bone meal	Fish solubles	Oatfeed
Bran	Gluten feed	Oatmeal
Brewers grains	Gluten meal	Oats
Brewers yeast	Gray shorts	Red dog
Cereal tailings	Hominy	Rice bran
Cheese meal	Iodinated casein	Soybeans
Corn	Linseed meal	Tomato pomace
Corn cob meal	Malt sprouts	Wheat
Corn flakes	Meat scraps	Whey
Cotton seed meal	Middlings	Urea
Distillers grains		

Minerals

Calcium carbonate	Iron oxide	Potassium iodide
Cobalt carbonate	Iron sulfate	Sodium chloride
Cobalt sulfate	Limestone	Sodium fluoride
Copper hydroxide	Manganese oxide	Sodium propionate
Copper oxide	Manganese sulfate	Sulfur
Copper sulfate	Phosphates (various types)	Zinc oxide
Iron carbonate		Zinc sulfate

Additives

2-acetyl-amino-5-nitrothiazole	Baciferm	Dihydrostreptomycin
Acetyl-(p-nitrophenyl) sulfanilamide	Bacitracin	2,2'-dihydroxy-5,5'-dichlorodiphenylmethane
Agrozyne	Biotin	Di-N-butyltin dilaurate
Aminonitrothiasole	Bithionol and methio-triazanime	3,5-dinitrobenzamide
Amprol	Cadmium anthranilate	Dinitrophenylsulfonylethylenediamine
Arsanilic acid	Chlorotetracycline	Dowzene
Arsensobenzene	Choline	Dried rumen bacteria
Ascorbic acid	2,4-diamino-5(p-chlorophenyl) 6-ethylpyrimidine	Dura bond
Aterrimin	Dienestrol diacetate	Dynafac
Aurofac 10	Diethylcarbamazine	
Aurofac 40	Diethylstilbestrol	

Enzymes	Nitrophanide	Pro-Gen
Erythromycin Thio- cynate	4-Nitrophenyl- arsonic acid	P-ureidobenzene- arsonic acid
Folic acid	3 Nitro powder	Reserpine
Furazolidine	Nystatin	Saccharin
Glycarbylamide	Oleandomycin chloro- form adduct	Santoguin
Hepside	Oxytetracycline	Silotracin
Histostat	Para aminobenzoic acid and sodium or potassium salts	Sodium arsanilate
Hydroxyzine hydro- chloride	Penicillin	Sodium chloride
Hygromix	Pepsin	Soybean hulls
Klotogen F	Phenothiazine	Streptomycin
Menadion sodium bisulfite	Piperazine	Sucro-Flavor
Methionine	Piperazine-dihydro- chloride	Sugar
Mycostati-20	Piperazine hexa- hydrate	Sulf-quinoxaline
Neomycin	Piperazine mono- hydrochloride	Terramycin
Neomycin sulfate	Piperazine phos- phate mono- hydrates	Vitamin A
Niacin	Piperazine sulfate	Vitamin B
Nicarbazin	Plant-sterols	Vitamin B <sub>6</sub>
Nicotine	Polystat	Vitamin B <sub>12</sub>
Nlearb		Vitamin D <sub>2</sub>
Nithiazide		Vitamin D <sub>3</sub>
Nitrofurazone		Vitamin E <sup>3</sup>
3-nitro-4-hydroxy- phenyl-arsonic acid		Vitamin K
		Wormal

#### APPENDIX V

Approximately 25 urine specimens were assayed each week for 18 weeks using a modification of an oral-feeding method of bioassay described by Watrous and Olsen.<sup>(3)</sup> Female Swiss ICR mice, 21 days of age, were given untreated urine or standard by gavage, 0.4 milliliter per day for 3 days. On the fourth day the mice were sacrificed, and the uteri were removed and weighed individually. Ten mice were used for each assay and the mean uterine weight compared to that of a control group receiving water only. A level of significance was reported for each assay.

The estrogenic activity of the urine specimens was determined from a dose-response curve prepared each week in the following manner. One water control sample and 2 to 7 samples containing known quantities of DES, ranging from 0.02 to 0.81 micrograms per milliliter, were assayed and the results plotted on semi-logarithmic graphic paper. Dose was plotted along the abscissa and mean uterine weights along the ordinate. Since DES was used as the standard and the bioassay is not specific for DES alone but measures endogenous estrogen and androgen as well, results are reported in DES equivalents.

#### Subcutaneous-Injection Method of Bioassay

Because the results by the oral-feeding method of bioassay proved to be quite variable, those urine specimens showing significant uterine responses (P less than 0.05) by this method were reassayed approximately six months later varying the procedure in the following manner. Female

Swiss ICR mice were ovariectomized at 21 days of age and given 0.2 milliliters of sample by subcutaneous injection twice daily for three days, starting on the day of ovariectomy. On the last day of injection the mice were autopsied and the mean uterine weight of the 10 mice compared to that of a control group as explained before. Estrogenic activity was determined from a dose-response curve prepared in the same manner using samples containing known quantities of DES.

#### Bioassay Statistical Procedures

The standard error of uterine weight was calculated as follows:

$$S.E. = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{N}}{N(N-1)}}$$

Where, x = uterine weight

N = number of mice used in one bioassay

For probability, the following formula was used:

Student's t (for use with probability table) =

$$\frac{M_1 - M_2}{\sqrt{(E_1)^2 + (E_2)^2}}$$

Where,  $M_1$  = average uterine weight for test group

$M_2$  = average uterine weight for control group

$E_1$  and  $E_2$  = S.E. for respective group

APPENDIX VI

The Results of the Laboratory Standardization Tests Showing the Mean Uterine Weight in Milligrams of Ten Mice and the Level of Significance According to the Week of Bioassay

Week of Bioassay	Control Mean uterine weight	Concentration of DES in Sample													
		0.02 µg/ml Mean uterine weight	P <sup>1</sup>	0.03 µg/ml Mean uterine weight	P	0.04 µg/ml Mean uterine weight		0.06 µg/ml Mean uterine weight		0.08 µg/ml Mean uterine weight		0.09 µg/ml Mean uterine weight		0.16 µg/ml Mean uterine weight	
11/22/60	12.4	13.4	NSD <sup>2</sup>			16.7	< 0.01								
11/29/60	13.5	14.3	NSD			18.6	< 0.01								
12/5/60	13.0	13.5	NSD			19.1	< 0.01								
12/12/60	15.0	16.3	NSD			19.7	< 0.01								
12/19/60	16.5	18.9	NSD			22.5	< 0.01								
12/29/60	17.5	17.8	NSD			22.2	< 0.01								
1/4/61	15.5	16.4	NSD			22.9	< 0.01								
1/10/61	17.2	16.4	NSD			22.1	< 0.01								
1/17/61	16.2			19.1	< 0.02							26.0	< 0.01		
1/23/61	15.4			19.3	< 0.02							28.8	< 0.01		
1/30/61	19.2	21.0	NSD			24.6	< 0.02								
2/6/61	18.5			19.3	NSD							28.7	< 0.01		
2/13/61	17.9			20.2	NSD							28.4	< 0.01		
2/20/61	17.2	18.5	NSD			22.4	< 0.01								
3/2/61	16.9	17.9	NSD			21.2	< 0.02			25.0	< 0.01			41.2	< 0.01
3/6/61	17.4	17.2	NSD			18.2	NSD			27.8	< 0.01			48.4	< 0.01
3/14/61	14.8	16.8	NSD			21.9	< 0.01			27.0	< 0.01			42.4	< 0.01
3/21/61	16.6	20.9	< 0.01			22.0	< 0.01			33.0	< 0.01			36.9	< 0.01
10/18/61 <sup>3</sup>	17.0	20.2	< 0.01	27.1	< 0.01	31.4	< 0.01	41.8	< 0.01			62.8	< 0.01		
10/24/61 <sup>3</sup>	16.1	17.8	NSD	24.0	< 0.01	27.7	< 0.01	41.8	< 0.01			62.8	< 0.01		
11/2/61 <sup>3</sup>	16.2	21.6	< 0.01	22.6	< 0.01	26.4	< 0.01	48.5	< 0.01			60.2	< 0.01		

- (1) Probability
- (2) No significant difference
- (3) Subcutaneous-injection method of bioassay

## APPENDIX VI - cont.

Week of Bioassay	Control Mean uterine weight	0.18 µg/ml		0.27 µg/ml		0.32 µg/ml		0.54 µg/ml		0.64 µg/ml		0.81 µg/ml	
		Mean uterine weight	P										
11/22/60	12.4												
11/29/60	13.5	23.0	<0.01					24.8	<0.01				
12/5/60	13.0	33.8	<0.01					64.9	<0.01				
12/12/60	15.0	41.2	<0.01					64.2	<0.01				
12/19/60	16.5	43.8	<0.01					61.8	<0.01				
12/29/60	17.5	40.0	<0.01					58.4	<0.01				
1/4/61	15.5	38.2	<0.01					56.5	<0.01				
1/10/61	17.2	42.2	<0.01					59.8	<0.01				
1/17/61	16.2			50.2	<0.01							56.8	<0.01
1/23/61	15.4			50.9	<0.01							62.0	<0.01
1/30/61	19.2	42.5	<0.01					65.0	<0.01				
2/6/61	18.5			53.3	<0.01							60.7	<0.01
2/13/61	17.9			54.6	<0.01							65.0	<0.01
2/20/61	17.2	50.8	<0.01					63.9	<0.01				
3/2/61	16.9					66.7	<0.01			60.2	<0.01		
3/6/61	17.4					59.1	<0.01			66.6	<0.01		
3/14/61	14.8					60.9	<0.01			66.2	<0.01		
3/21/61	16.6					55.6	<0.01			56.7	<0.01		
10/19/61 <sup>3</sup>	17.0												
10/24/61 <sup>3</sup>	16.1												
11/2/61 <sup>3</sup>	16.2												

APPENDIX VII

Results of 48 Field Standardization Tests Performed on 24 Divided Urine Specimens

Urine Specimen Number	Divided	DES added (micrograms per ml.)	Oral-feed Method			Subcutaneous-injection Method		
			Week of Test	DES Equivalents (micrograms per ml.)	Probability	Week of Test	DES Equivalents (micrograms per ml.)	Probability
1	a	None	11/22/60	Negative	NSD*	—	—	—
	b	None	12/5/60	Negative	NSD	—	—	—
2	a	None	1/4/61	Negative	NSD	—	—	—
	b	None	3/6/61	Negative	NSD	—	—	—
3	a	None	1/30/61	Negative	NSD	—	—	—
	b	None	3/6/61	Negative	NSD	—	—	—
4	a	None	2/6/61	Negative	NSD	—	—	—
	b	None	3/6/61	Negative	NSD	—	—	—
5	a	None	3/2/61	Negative	NSD	—	—	—
	b	None	3/2/61	Negative	NSD	—	—	—
6	a	None	2/13/61	Negative	NSD	—	—	—
	b	None	3/6/61	Negative	NSD	—	—	—
7	a	None	11/29/60	0.048	< 0.01	10/18/61	Negative	NSD
	b	None	12/5/60	Negative	NSD	10/18/61	Negative	NSD
8	a	None	12/19/60	Negative	NSD	10/18/61	Negative	NSD
	b	None	12/19/60	Negative	NSD	10/18/61	Negative	NSD
9	a	None	12/19/60	Negative	NSD	10/18/61	c.0.004	< 0.05
	b	None	12/19/61	0.024	< 0.05	10/18/61	Negative	NSD
10	a	None	1/23/61	Negative	NSD	10/25/61	Negative	NSD
	b	None	2/20/61	Negative	NSD	10/25/61	c.0.030	< 0.01
11	a	None	2/13/61	0.035	< 0.02	10/25/61	c.0.015	< 0.05
	b	None	3/6/61	Negative	NSD	10/25/61	Negative	NSD

\* No significant difference

## APPENDIX VII - cont.

Urine Specimen Number	Divided	DES added (micrograms per ml.)	Oral-feed Method			Subcutaneous-injection method		
			Week of Test	DES Equivalents (micrograms per ml.)	Probability	Week of Test	DES Equivalents (micrograms per ml.)	Probability
12	a	None	1/10/61	Negative	NSD	10/25/61	Negative	NSD
	b	None	3/6/61	Negative	NSD	10/25/61	Negative	NSD
13	a	None	11/29/60	0.028	< 0.05	10/18/61	Negative	NSD
	b	0.480	12/12/60	0.540	< 0.01	--	--	--
14	a	None	12/5/60	Negative	NSD	--	--	--
	b	0.390	12/12/60	0.320	< 0.01	--	--	--
15	a	None	12/19/60	Negative	NSD	--	--	--
	b	0.400	12/19/60	0.470	< 0.01	--	--	--
16	a	None	1/10/61	Negative	NSD	--	--	--
	b	0.200	2/20/61	0.142	< 0.01	--	--	--
17	a	None	1/30/61	Negative	NSD	--	--	--
	b	0.100	3/6/61	0.104	< 0.01	--	--	--
18	a	None	1/30/61	Negative	NSD	--	--	--
	b	0.100	1/14/61	0.088	< 0.01	--	--	--
19	a	None	1/10/61	Negative	NSD	--	--	--
	b	0.200	3/6/61	0.182	< 0.01	--	--	--
20	a	None	2/20/61	Negative	NSD	--	--	--
	b	0.200	3/2/61	0.270	< 0.01	10/25/61	c.0.169	< 0.01
21	a	None	12/19/60	Negative	NSD	10/18/61	c.0.003	c.0.05
	b	0.420	12/19/60	0.240	< 0.01	10/18/61	c.0.183	< 0.01
22	a	None	1/10/61	Negative	NSD	10/25/61	Negative	NSD
	b	0.200	3/6/61	0.130	< 0.01	10/25/61	0.156	< 0.01
23	a	None	2/13/61	Negative	NSD	10/25/61	c.0.008	< 0.05
	b	0.100	3/14/61	0.174	< 0.01	10/25/61	c.0.100	< 0.01
24	a	None	3/2/61	Negative	NSD	10/25/61	Negative	NSD
	b	0.100	3/2/61	0.185	< 0.01	10/25/61	c.0.105	< 0.01

APPENDIX VIII

The following respirators are applicable to likely exposures in animal feed mills: (27)

Toxic Dust Respirators

These respirators are approved for protection against the inhalation of toxic metallic dusts that are not significantly more toxic than lead, such as lead, cadmium, arsenic, chromium, manganese, selenium, vanadium and their compounds.

These respirators are NOT approved for protection against fumes generated by heating and volatilization, such as fumes generated in lead burning and soldering, smelting, type founding, and fusing lead glaze and enamel.

1. Willson Rotiform Respirator No. 200L. Approval BM-2123
2. A.O.\* R9100-T Respirator. Approval BM-2144
3. A.O.\* R1016 and R-2016 Respirators. Approval BM-2138

Dust Respirators

These respirators formerly were designated "Type A and Lead Dust," and "All-Dust." They are approved only for protection against the inhalation of dusts that are not significantly more toxic than lead, such as arsenic, asbestos, cadmium, chromium, lead, manganese, selenium, silica, vanadium, and their compounds, and nuisance dusts.

1. Welsh No. 7100 Respirator. Approval BM-2175
2. A.O.\* R5090 Respirator. Approval BM-2174

3. Pulmosan C-264 Respirator. Approval BM-2173
4. Cesco Healthguard No. 94 Respirator. Approval BM-2124
5. Cover Dupor No. 46 Respirator. Approval BM-2124
6. Willson Monomask Respirator No. 600. Approval BM-2170
7. M.S.A.\*\* Comfo Respirator with Type "F" filters. Approval BM-2168
8. McDonald Dustfoe #66 Respirator. Extension of Approved BM-2166
9. A.O.\* R-2090 Respirator. Approval BM-2172
10. McDonald Clear-Vue Dustfoe Respirator. Approval BM-2148
11. M.S.A.\*\* Clear-Vue Dustfoe Respirator. Extension of Approval BM-2148
12. Willson Respirator No. 750DA. Approval BM-2151
13. Willson Respirators Nos. 5D and 45D. Approval BM-2152
14. A.O.\* R2000 Respirator. Approval BM-2154
15. A.O.\* R5050 and R5050A Respirators. Approval BM-2156
16. Willson Respirators Nos. 6 and 460. Approval BM-2161
17. Willson Respirator No. 860. Approval BM-2162
18. DeVilbiss Respirator MSD-5-5. Extension of Approval BM-2154
19. DeVilbiss Respirator MSE-501. Extension of Approval BM-2156
20. Pulmosan L-3100 Pouch-type Respirator. Approval BM-2165
21. Pulmosan L-800 Respirator. Approval BM-2160
22. A.O.\* R1017 and R2017 Respirators. Extension of Approval BM-2138
23. Cesco No. 96 Respirator. Approval BM-2167
24. M.S.A.\*\* Dustfoe #66 Respirator. Extension of Approval BM-2166
25. M.S.A.\*\* Ltd. "Dustfoe #55" Respirator. Extension of Approval BM-2166
26. Pulmosan L-801 Respirator. Approval BM-21A-76

\* American Optical Company

\*\* Mine Safety Appliances Company

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