GRANT-IN-AID-PROPOSAL

ELIMINATION OF POLYCHLORINATED BIPHENYL RESIDUES FROM SILOS

submitted to

Monsanto Industrial Chemicals Company
St. Louis, Missouri

by

Ohio Agricultural Research and Development Center

Wooster, Ohio

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TABLE OF CONTENTS

	Page
JUSTIFICATION	2
PREVIOUS WORK AND PRESENT OUTLOOK	5
OBJECTIVES	11
TECHNICAL APPROACH	12
Silo Coating Material Experiments	12
Aroclor Feeding Experiments	14
ANALYTICAL PROCEDURES	17
QUALIFICATIONS	19
Personnel and Responsibilities	19
Current PCB and Related Research	19
Facilities and Equipment	22
PROPOSED TIMETABLE	27
PROPOSED BUDGET	28
LITERATURE CITED	29
APPENDIX	

MONS 042593

ELIMINATION OF POLYCHLORINATED BIPHENYL RESIDUES FROM SILOS

A large number of silos throughout Ohio and neighboring states are contaminated with polychlorinated biphenyl residues. These residues in turn contaminate the silage and the products produced by livestock that consume this silage. Since polychlorinated biphenyls are an illegal contaminant of human foods, livestock producers with contaminated silos are faced with and/or have suffered substantial financial losses from unmarketable produce and silo replacement.

In 1971 studies were begun in the Department of Dairy Science of the Ohio Agricultural Research and Development Center to eliminate polychlorinated biphenyl residues from producing dairy cows and their feed supply. The three approaches to the problem have been: to increase the rate of PCB clearance from the animal; to remove the milk fat and associated PCB from non-fat milk components; and to develop coating systems to permanently cover the PCB contamination in the silo. The research included in this proposal represents a continuation of this effort to eliminate these residues from livestock products.

MONS 042594

JUSTIFICATION

Polychlorinated biphenyls (PCB's) have recently been detected in livestock feedstuffs and subsequently in meat, eggs, poultry, fish and milk. These compounds, as are all synthetic chlorinate hydrocarbons, are illegal contaminants of human food. The discovery of these compounds has caused considerable concern among those associated with animal agriculture and substantial financial losses to those who have been directly affected by this contaminant.

A major source of PCB contamination of milk and meat has been silos which were coated to prevent leakage and erosion of the walls with a material that contained the Aroclor's[1] 1254 and 5460. Those PCB compounds were not specifically produced for incorporation into silo coatings, but were used because they are good plasticizers.

Reports of silos contaminated with PCB's have come from a number of midwestern, mideastern and southern states. The exact number of PCB contaminated silos is not known, however, it is suspected that over 200 silos in the Ohio-Michigan area are so contaminated. The contamination in these silos is a chronic problem over many years. Silos coated as many as 16 years ago with the Aroclor containing coating still contain substantial quantities of the PCB.

Currently only the Ohio State Department of Agriculture is conducting a regular testing program to detect and ban the

MONS 042595

[1]Aroclor is trademark of Monsanto Chemical Co.

shipment of milk that contains more than 5.0 ppm of Aroclor on a fat basis. However, budget and personnel shortages within this Department have prevented routine testing of all producers. Twelve producers in Ohio have had to cease milk shipment until the levels of Aroclor decreased to less than the "actionable level."

This problem will no doubt affect increasing numbers of dairymen and cattlemen in the near future. State and federal agencies will attain the capabilities to assay these hydrocarbons and more producers will have their milk and meat products checked for PCB contamination. Furthermore, the Food and Drug Administration has proposed lowering the "administrative guidelines" and reclassifying them as "temporary tolerances." If this proposed change occurs then the current allowable tolerance of 5 ppm for milk on fat basis (equal to 0.2 ppm whole milk basis if milk has 4% fat) would be reduced by half to 2.5 ppm on fat basis. If this change should occur then many livestock producers who formerly were below the allowable tolerance would be subject to a shipment ban on their products.

A livestock producer who has a silo contaminated with PCB's is faced with considerable financial loss. Replacement of the basic silo structure will probably cost in excess of $4,000.00. If his milk is unmarketable he may be losing $6.50 per hundred pounds rejected and contaminated, unmarketable livestock may have been worth $36.00 per hundred pounds. Therefore, it seems

justifiable that an effort be made to develop low cost procedures that will reduce the level of the PCB contamination from silos below that which may cause rejection of the farm product.

PREVIOUS WORK AND PRESENT OUTLOOK

Description of the distribution, and some biological effects of PCB's have been reviewed (7, 10, 12, 13). There is no need to review or discuss these general aspects of PCB contamination here. The following discussion will be limited to the contamination of milk and silage.

When dairy cows were fed Aroclor 1254, or a feed contaminated with this chlorinated hydrocarbon, residues were deposited in the body fat and secreted with milk fat (3, 5). This residue may appear in the milk within 12 hours (unpublished results from our laboratory) and when fed continually approached a "steady state" in about 40 to 60 days (1, 5). Fries et al. (5) fed 200 mg of Aroclor 1254 daily to nine cows for 60 days. The average excretion of Aroclor residue between the 40th and 60th day was 42.3 mg per day which accounted for 21% of the intake. As yet it is not known if the rate of excretion into milk is linearly related to the concentration of Aroclor consumed.

When Aroclor feeding was discontinued a rapid decline in the residue in milk was observed with about a 50% decline in residue concentration in 15 days (5). Thereafter, the rate of decline was slower being 1.0 to 1.5% per day (4, 5).

Polychlorinated biphenyls have been shown to have a similar rate of elimination from cows as do some pesticides (3, 4), especially DDE (6). The rate of dieldrin excretion was increased by feeding phenobarbital or activated carbon (17). Unfortunately, PCB excretion was not increased by feeding these compounds

(1, 4). Acceleration of PCB excretion in cows has not yet been achieved; however, studies are in progress in our laboratory utilizing high-fat diets. This approach seems promising since Rusoff et al. (14) accelerated removal of DDT and dieldrin from contaminated cows by feeding high fat diets. This accelerated removal of the chlorinated hydrocarbons was probably the result of increased turnover of the body adipose tissue. Palmquist and Conrad (9) have shown that a high fat diet may reduce the turnover time of the body adipose tissue pool by 30%.

The mechanism by which PCB's from Aroclor-containing coatings contaminate the silage was originally thought to be only mechanical by flaking off of contaminated chips of the coating. It is not uncommon for these chips to contain 5,000-10,000 ppm of Aroclor. However, recent work has shown that the Aroclor is soluble in silage juice (15). The solute may well be acetic acid, one of the important organic acids formed when the silage ferments. Aroclor 1254 has been shown to be soluble in acetic acid (8). The Aroclor found in the silo walls and in the silage does not appear to be different than Aroclor standards when analyzed by gas chromatography (1, 15). This suggests little or no microbial degradation of Aroclor 1254 during the ensiling process (2).

Skrentry et al. (15) reported that the majority of the Aroclor was detected in the six inches of silage adjacent to the silo wall. However, the contamination diffused through

the silage and was detectable 48 inches from the silo wall. Similar results were obtained in our studies, except no contamination was detected beyond 36 inches from the silo wall (16).

In the aforementioned study by Willett (16) the use of certain coating materials to cover Aroclor contamination in a silo and on stave blocks substantially reduced the Aroclor levels in silage. These studies indicated that the thickness of the coating and the solvent carrier used were important factors influencing the quantity of Aroclor residue found in the silage.

By utilizing the data in these previously discussed studies it appears that the recoating of silos that are contaminated with Aroclor may well be a practical means of reducing the contamination to below the proposed maximal tolerance levels. The following example illustrates how this may be a feasible approach.

Figure 1 is derived from the data of Willett (16) and shows the mean concentrations of Aroclor 1254 that appeared in the silage (dry matter basis) at various distances from the silo wall. These data are from the coating test sections of a silo with an 8-foot diameter. Also shown is the approximate percentage of the total silage which is between the various sampling distances from the wall. By utilizing these percentages and the mean concentrations of Aroclor within each distance from the silo wall a weighted mean concentration of Aroclor

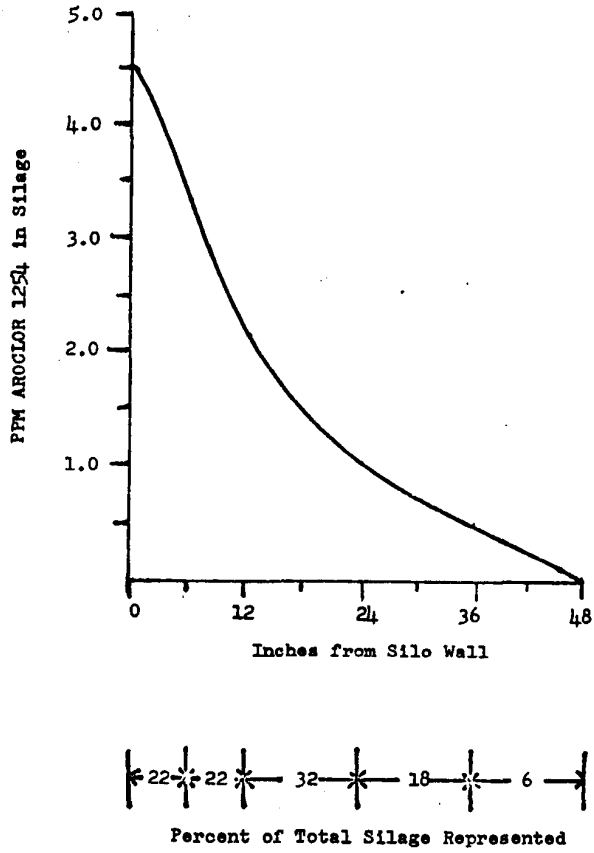

FIG. 1. Mean concentrations of Aroclor 1254 that appeared in silage (dry matter basis) at various distances from the silo wall. The lower diagram represents the approximate percentage of the total silage between the various sampling distances from the wall.

can be calculated for the blended silage from the entire silo. This gives us a meaningful figure with which to work, since on commercial dairy farms, silo unloaders discharge silage from all parts of the silo.

For this example we will presume that a cow is being fed 13.6 kg of silage daily, and the silage is 33.3% dry matter. We will also presume that the cow is producing 18.6 kg of milk daily which is the average production of cows in Ohio on DHIA testing. Utilizing weighted mean concentration of Aroclor from Figure 1, the cow in our example would consume 8.8 mg of Aroclor daily. If 21% of this dose is excreted in the milk, as has been reported by Fries et al. (5), then 1.87 mg or 0.1 ppm would be in the milk daily.

The estimate of the concentration of Aroclor in milk for this example would be a maximal figure. First, development of a coating system that would reduce the level of contamination of silage adjacent to the silo wall to 2.0 ppm or less seems very possible. Second, the majority of the silos in use today are of larger diameter than 8 feet thus the contaminant would be diluted more.

Additional research is necessary to establish two components of the above example. Further testing of coating materials is necessary to verify their effectiveness as barriers to the PCB's in the silo walls. Also, further verification of the quantity of the consumed dose of Aroclor that appears in milk is necessary. Particularly the percent of the consumed dose

appearing in milk must be established when low levels (1-20 mg per day) of Arcolor are fed.

MONS 042603

OBJECTIVES

1. To determine how effectively various coating materials prevent contamination of silage with Aroclor 1254 from previously used silo coatings.

2. To determine the durability of various coating materials when used in a silo to cover PCB contamination.

3. To determine the concentration of residue from Aroclor 1254 in milk resulting from known concentrations of Aroclor 1254 in the silage.

Although this might be good info to have, I believe the primary objective is to eliminate PCB contamination of silage. Hence, this info. is sorta academic.

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TECHNICAL APPROACH

In order to achieve the outlined objectives, four approaches will be used. Three experiments will evaluate coating materials for silos and one will determine the concentration of residue from Arocolor 1254 in milk resulting from known levels of intake.

Criteria for the selection of coating materials to be tested were: The effectiveness of certain types of coatings in the preliminary study (15); the physical and chemical properties of the coating (thickness, solvents, etc.); and, the cost of the material. Only materials that would cost less than $600.00 for purchase and application (14' x 50' silo) are used.

The levels of Arocolor for the feeding experiment are designed to approximate trace contamination of silage yet maintain the milk residue at 0.1 ppm and less.

Silo Coating Material Experiments.

1. Silo stave block experiments. Oumar is the name of a coating used by several midwestern silo companies and is responsible for most, if not all, of the Arocolor contaminated silos. This coating contains 70 lb. Arocolor 1254 and 20 lb. Arocolor 5460 per 47 gallons of paint. It should not be confused with Neville R-1-6A resin that has the same name. What's the difference

In these studies concrete silo stave blocks will be coated with Oumar. After the Oumar has had adequate time to cure then the various experimental coatings will be applied to cover this what are they?

Aroclor containing material. Untreated and Cumar coated blocks will be maintained as controls.

The blocks will be placed in polyethylene lined 55 gallon drums, chopped corn or alfalfa will be pressed into the drums and sealed for four months. During this period the ensiling process will take place in the same manner as in a large silo. Silage samples will be collected at three depths and three distances from the blocks and analyzed for Aroclor 1254.

These block studies provide the opportunity to rapidly and at low cost evaluate the performance of the coatings as barriers to Aroclor contamination of silage. These studies will provide for minimal extraneous contamination and sampling can be very accurate. Unfortunately the stave block experiments do not evaluate the durability of the coating when used in an actual silo.

2. Experimental silo. A silo which had a residue of over 600 ppm of Aroclor in scrapings of the wall will be used as the test structure. This silo was divided into test sections with Masonite sheets, the walls were wire brushed to provide a sound clean surface and various coatings were applied. See the report by Willett (16) for additional details.

These experiments will be conducted by filling the silo to the top of the test sections with chopped corn or alfalfa and allowing normal ensiling to take place. Four months after filling, the silage will be sampled at specific depths and distances from the wall. These will be analyzed for Aroclor to determine

the vertical and horizontal distribution of the residue.

This silo will be serially filled and sampled. This will test the durability of the various coatings as well as determine whether the coating maintains an effective barrier to Aroclor when used continually.

3. Field silos. Some silos that are known to be contaminated with Aroclor will be entirely coated with the materials that appeared most effective in our preliminary study (16). These will be silos that are in use on commercial dairy farms. Prior to recoating of these silos, samples of silage, silo wall scrapings and milk from the bulk tank will be analyzed for Aroclor. Following filling, biweekly samples of silage will be collected adjacent to the silo wall. On the same day that the silage is collected samples of milk from the bulk tank will also be collected. All samples will be analyzed for Aroclor.

Presently, one silo is on experiment and another will be coated in August 1972. The number of silos that will be involved in this phase of the research is not known because the dairyman must volunteer to have his silo used, must stand all costs and assume all risks.

Aroclor Feeding Experiments.

In order to determine the amount of Aroclor residue that appears in the milk from a known daily intake, 70 day feeding trials will be conducted. Initially six animals will be allotted to one of three levels of daily intake of Aroclor. These Aroclor feeding levels will be 14.0 mg, 7.0 mg, and 3.5 mg

daily. If the results from these animals are highly variable, additional animals will need to be employed.

All animals will be on a standard feeding program and will be fed corn silage, alfalfa hay and grain concentrate at the ratio of 1:1:1 on the as fed basis. The Aroclor will be fed once-daily by adding a premeasured acetone dilution to the silage. The acetone will be allowed to evaporate prior to feeding. Refusal (if any) will be weighed and analyzed for Aroclor so that the intake can be corrected.

Milk will be sampled from each animal biweekly and analyzed for Aroclor residue. Weekly samples of urine and feces will also be collected and analyzed.

Data from these animals will be analyzed to determine the percentage of the consumed dose that appears in the milk. Other analysis will determine: If parallelism occurs between the consumed dose and levels of residue in the milk; the time required to reach biological equilibrium; and, variability among animals. Levels of Aroclor residue in urine and feces should further substantiate the proportions of this hydrocarbon cleared by these excretory pathways.

Since unverified reports claim reproductive failure and calfhood mortality among cows fed Aroclor contaminated silage, accurate records of the reproductive performance of the animals will be kept during the Aroclor feeding periods. Feeding of such low levels of Aroclor are not expected to alter the reproductive pattern.

All experimental animals that die or are killed will be necropsied and selected tissues analyzed for Aroclor residue accumulation.

ANALYTICAL PROCEDURES

The facilities and equipment available for these studies are discussed in the "Qualifications" section of this proposal and will not be repeated here. The procedure for the extraction of silage is in the appendix. Milk, urine and feces samples are also conducted by this method with only slight modifications.

The extraction procedure (unpublished); consists of an acetone extraction, partitioning against petroleum ether and a clean up on a silica gel-florsil column; and was developed by A. C. Waldron with modifications by L. B. Willett. For silage, this method has provided very repeatable results, low backgrounds and consistent recovery of known standards. The mean background level for silage samples has been 0.020 ppm. The mean recovery from standards (between 0.05 and 2.0 ppm) was 67.45 $\pm$ 1.31%.

The procedure has provided very low background levels (0.009 ppm) and good repeatability for the extraction of milk samples. However, the percent recovery is not constant between 0.05 and 1.0 ppm. Instead there is a repeatable loss of about 0.045 ppm from all standards between 0.1 and 1.0 ppm. This extraction procedure has not proven dependable on standards with a concentration of 0.05 ppm. For this reason, Aroclor levels will be determined on milk fat samples rather than whole milk.

Gas chromatography of samples is conducted on an F & M 402 with a ^{63}Ni electron capture detector. The column is an 8 foot by 1/8 inch and packed with 4% DC-200 and 6% QF-1 on Gas Chrom Q

(80-100 mesh). The running conditions are: Column 210°, detector 300 and Argon-Methane flow 42 ml per min. A typical standard tracing is enclosed in the appendix.

Quantitation of the sample is done by disc integrations and comparison of these to the integrations of known standards. Care is taken to maintain sample concentration within the linear range of the detector. For silage samples total integrations from peak "F" through peak "M" (see appendix) are used, while for milk the integrations from peak "D" through peak "P" are used.

Silage samples are extracted on a wet basis as they come from the silo. The Aroclor concentrations in silage samples are expressed on a dry matter basis to remove variability due to moisture. Dry matters are determined on a portion of the sample by weighing before and after drying at 100° C for 48 hours. Aroclor levels in milk are reported both on a whole milk and fat basis. Milk fat percent is determined by the TESA method.

QUALIFICATIONS

PERSONNEL AND RESPONSIBILITIES.

The proposed research program will be conducted by personnel of the Ohio Agricultural Research and Development Center, departments of Dairy Science and Entomology (Pesticides Analytical Laboratory). The personnel, their departments, fields of speciality and responsibilities to the project are listed below. Biographical resumes are included in the Appendix.

Dr. L. B. Willett. Dairy Science, Physiology, and dairy management. Dr. Willett will be the project leader. He will direct and conduct the silo and animal phases of the studies. He will also direct most of the Aroclor analyses.

Dr. A. C. Waldron. Entomology. Pesticide and PCB analysis. Dr. Waldron will conduct a portion of the Aroclor analyses. He will also assist with the interpretation of PCB values obtained.

Dr. H. R. Conrad. Dairy Science. Ruminant Nutrition. Dr. Conrad will direct the nutritional aspects of the proposed studies.

CURRENT PCB AND RELATED RESEARCH.

Presently, studies are being conducted cooperatively between the departments of Dairy Science and Entomology (Pesticides Analytical Laboratory) to develop methods by which dairy systems can be decontaminated of PCB residues which originate from

coatings used to seal silos which contain Aroclor 1254. There are four objectives of the current research.

First, to study methods of and recommend a procedure for decontaminating silos contaminated with PCB. Second, to identify dietary agents abetting the removal of PCB from dairy cows. Third, to develop a feasible approach for selectively skimming milk from highly contaminated cows to reduce PCB contamination of the total milk supply to below the actionable level. Fourth, to determine any pathological or toxicological changes and PCB levels in the body tissues of PCB-contaminated animals that die or are killed.

Two lines of investigation have been used in the study of silo decontamination. A silo, which is known to contain a substantial quantity of PCB in its protective coating, is used as the major structure for these studies. The silo has been sectioned into test blocks which were mechanically coated with solvent- and water-based materials that are considered impervious to the Aroclor in the silo walls. Six to eight months after the silo was filled the effectiveness of the cover coatings were determined by analyzing the silage for Aroclor. A copy of the preliminary results is included in the Appendix.

In other studies concrete silo blocks have been painted with a coating that contains Aroclor 1254. Then the blocks have been recoated with the materials designed to prevent Aroclor migration. These blocks were placed in air tight drums of silage for four months. The silage has been analyzed for Aroclor

contamination.

Experiments with 10 coating materials have been completed. Further experiments are in progress utilizing those coatings which appear most effective plus new coatings with the properties that seem to form good barriers to PCB contamination.

A series of experiments are presently in progress to determine the biological behavior of a single oral dose of Aroclor 1254 given to cows fed various dietary regimes. This study involved a comparison of the rate of Aroclor excretion in milk, urine and feces when the cows were fed high-grain and high-fat diets versus those fed normal diets. It is known that the turnover of fat in cows occurs at a more rapid rate when a high-fat ration is fed compared with the turnover of fat in animals receiving normal amounts of roughages. Conversely, high-grain rations reduce the turnover rate of adipose tissue.

The determination of Aroclor that appears in the feces indicates the percentage of a single three gram Aroclor dose that was absorbed from the gut. Urinary Aroclor levels show the importance of this excretory route. The Aroclor level in milk indicates the amount of Aroclor excreted in milk, the rate of excretion, the time required for the milk level of Aroclor to fall below the actionable level (0.2ppm and/or 0.1ppm) and the influence of high-fat and high-grain rations on the aforementioned excretion of Aroclor into milk. The results of these studies are not yet available as data analysis has not been completed.

An effort has been made to develop a system of management procedures for skimming milk centrifugally in order to remove the fat portion of the milk and the PCB contaminants. In this study milk from contaminated cows as well as milk contaminated with known quantities of Aroclor were assayed for levels of PCB. Milk was then subjected to centrifugal separation of the milk fat. The skimmilk and fat portions were then reassayed for PCB levels. The results of this study agree with those of Platonow et al. (11) which show that nearly all of the PCB is associated with the milk fat and can be separated from the skimmilk.

Aside from the PCB analyses conducted for the above studies, research at the Pesticides Analytical Laboratory includes: analyses of PCB and organochlorine, organophosphate and carbamate insecticides, and various herbicides in lake and river drainage water; analyses of milk and feed products for DDT and dieldrin residues; and several projects concerning residues in fruit vegetables and animal tissues.

FACILITIES AND EQUIPMENT.

The Ohio Agricultural Research and Development Center is charged with the responsibility of conducting basic and applied research in agriculture and home economics and their related fields. It also has the responsibility of dissemination of new knowledge for the benefit of all segments of our society, and of training graduate students in cooperation with The Ohio State University Graduate School.

The O.A.R.D.C. is a separate legal and budgetary entity and is governed by its own Board of Control. The professional staff of the 14 subject matter departments includes about 240 scientists, including 120 faculty members at The Ohio State University who devote part-time to O.A.R.D.C. research. This effort is supported by about 185 technicians and research assistants, 295 civil service employees, and 120 temporary seasonal workers. Some 500 people are employed at Wooster during summer and about 415 during other seasons of the year.

One of the newest additions to the O.A.R.D.C. research program is the Laboratory for Environmental Studies, established in June 1970. The new laboratory coordinates environmental research and initiates additional studies on pollution and improvement of the environment.

A 70,000 volume research library on campus is housed in a new tri-purpose building. This facility has the library, a 1000 seat auditorium, and the statistical laboratory. The latter facility is equipped with IBM 1130 computer and has the necessary supporting accessory equipment. The statistical laboratory has a statistician for assistance in statistical analyses. A photography laboratory, staffed by two experienced photographers, and print shop services are available to O.A.R.D.C. personnel. Trained veterinarians in the Veterinary Science Department provide animal health services and surgical procedures when necessary. Experimental rations are prepared at the O.A.R.D.C. feed processing facility, having micro and macro mixing capabilities.

which can provide feedstuffs in a variety of forms including pellets.

The Dairy Science Department has 110 cow milking herd which consists of both Jerseys and Holsteins. The young female animals are reared at the Research Center for experimental purposes and as herd replacements. The dairy herd is used for studies in milk secretion, nutrition and physiology, herd management, and environmental quality. The North Central Branch of O.A.R.D.C. has a herd of 100 registered Holsteins which are used for nutritional and breeding studies.

The dairy farm unit has facilities to handle cows in groups, as individuals or in isolation. Facilities and procedures are routinely used whereby experimental animals are individually fed, milked and urine and feces collected. Laboratories are available at the dairy unit with facilities to determine dry matter of feed and feces samples as well as facilities for the preparation of samples for storage. These dairy facilities will be used for the proposed research.

Experienced technical support is presently employed at the dairy unit to do the feeding, milking, Aroclor administration and sample collection. Cows for the proposed experiment will be obtained from the O.A.R.D.C. herd.

Three laboratories in the Dairy Science Department are devoted entirely or in part to the analysis of PCB residues. The PCB extraction laboratory (300 sq. ft.) is complete with tables,

glassware, chromatography columns, solvent distillation apparatus and storage space. The gas-chromatography laboratory (300 sq. ft.) is equipped with two F and M Model 402 gas-chromatographs, one of which is equipped with Ni^{63} electron capture (EC) detection. Also available is a Varian Aerograph Hi Fi gas-chromatograph. The F and M 402 with the EC detector is committed to PCB studies. The analytical chemistry laboratory (1000 sq. ft.) is also used for some phases of PCB analysis and for protein, mineral and vitamin A analyses. This laboratory is also complete with tables and glassware including: Kjeldahl apparatus; fraction collector; electrophloresis unit; colorimeter; refrigerated centrifuge; auto-analyzer for nitrogen, urea, calcium, phosphorus, blood sugar and protein-bound iodine; and separate rooms for handling and counting radioactive materials, amino acid analysis and analytical weighing. Walk-in cooler and freezer space as well as freeze drying equipment are available for preserving samples.

The Pesticides Analytical Laboratory, in the Entomology Department, will conduct a portion of the PCB analyses. This laboratory has been in operation since 1965, and is under the direction of Dr. A. C. Waldron. In this laboratory a wide range of pesticides, herbicides and PCB's have been studied in water, milk, eggs, plants and animal tissues.

The Pesticides Analytical Laboratory is complete with tables and vacuum evaporators, ovens, water baths, bulances, mixers, grinders and appropriate glassware required for the analysis of PCB residues. Also available is a Barber-Colman Series 5000

gas-chromatograph with electron capture detector and a Beckman "B" spectrophotometer. Additional equipment available to the laboratory include: Dohrmann microcoulometer detector C-200-A with chloride and sulfur capture detector, F and M gas chromatograph with EC detector, Beckman DU-2 spectrophotometer with spectral fluorescence attachment, Beckman IR-10 infrared spectrometer and a Kontes Sweep co-distillation apparatus.

MONS 042619

PROPOSED TIMETABLE

If full time technical assistance can be hired by October 1, 1972, the following timetable should be possible:

January 1, 1973 - Data available from second silo trial and from second stave block trial. Complete data should be available from first filling of one field silo. Partial data should be available on the Aroclor feeding study.

April 1, 1973 - Data available on the third silo trial and the Aroclor feeding study. Partial data should be available on continued used of the field silos and third stave block trial.

July 1, 1973 - All data should be available for final analysis unless previously done.

October 1, 1973 - All aspects of the outlined research should be completed.

MONS 042620

PROPOSED BUDGET

Elimination of Polychlorinated Biphenyl Residues from Silos

The following Proposed Budget will cover the direct costs and administrative overhead for the PCB studies described in this proposal. This Proposed Budget includes the costs that will be incurred between October 1, 1972 and September 30, 1973.

Labor Costs:		
Technical Assistant (Temporary) 12 month employment	$7,296.00	
Student Labor (Part-time) To do dishwashing, etc.	600.00	
TOTAL LABOR		7,896.00
Supplies:		
Solvents for extraction	423.00	
Chromatography reagents	811.00	
Misc. glassware and reagents	300.00	
Gas-chromatography gas	320.00	
Gas-chromatography service & supplies	250.00	
TOTAL SUPPLIES		2,104.00
Administrative Overhead:		2,500.00
GRAND TOTAL		$12,500.00

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MONS 042622

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MONS 042623

APPENDIX

PCB Extraction of Silage

1. Grind sample until largest particles are 0.5 cm in length.
2. Weigh 50 g of the silage sample and place in 500 ml flask.
3. Extract with 250 ml redistilled Acetone by shaking for 12 hours on shaker.
4. Filter flask contents through Whatman's #1 filter paper into a 500 ml graduate cylinder.
5. Record the volume of the Acetone extract.
6. Put the entire acetone extract volume in a separatory funnel and add 500 ml of 2% Na_2SO_4.
7. Extract the above phase 1x with 200 ml redistilled pet ether (30° - 60° C) and then again 1x with 150 ml redistilled pet ether.
8. Combine both pet ether extracts and wash with 100 ml of 2% $NaHCO_3$.
9. Dry the pet ether on rotary evaporator 42° C to a volume of about 20 ml.
10. Prepare a chromatography column as follows:
    a. Put glass wool in bottom
    b. Put in 2½ inches of Florsil 60/100 mesh (Activated)
    c. Put in 5 inches of Silica Gel (Davidson 923) (Activated)
    d. Put in 1 inch of Na_2SO_4 (Anhydrous)
    e. Wash column with 100 ml of pet ether
11. Add sample (in the + 20 ml pet ether) to the column. When the sample is completely absorbed in the column, elute it with
    a. 250 ml of 2% Benzene in pet ether - elutes PCB's
    b. 250 ml of Benzene
12. Dry down on rotary to about 10 ml and transfer into vials for storage with eluting solvent.

Biographical Sketch

Name: Willett, Lynn B. Birth Date: 2 Aug. 1944

Title: Ass't. Prof. Nationality: U.S. Sex: Male

Birthplace: Colorado Springs, Colo.

Education:

Institution	Location	Degree	Year
Colo. State Univ.	Fort Collins	B.S.	1966
Purdue Univ.	Lafayette, Ind.	M.S.	1968
Purdue Univ.	Lafayette, Ind.	Ph.D.	1971

Honors:

Sigma Xi

Major Research Interest:

a. General interests are the physiology and management of dairy cattle.
b. Specific interests are the decontamination of dairy systems of PCB's, and steroid physiology with respect to environmental adaptation.

Research and Professional Experience:

1. Graduate Research Assistant
   Department of Animal Sciences
   Purdue University, Lafayette, Ind. 1966-1971

2. Assistant Professor, Research
   Department of Dairy Science
   Ohio Agricultural Research and Development Center
   Wooster, Ohio 1971-present

Selected References:

1) Willett, L. B. and Albright, J. L. Dairy Management in Larger Herds. J. Dairy Sci., 51: 138. 1968.

2) Willett, L. B. Albright, J. L. and Cunningham, M. D. Once- Versus Twice-Daily Feeding of Milk Replacer to Calves. J. Dairy Sci., 52: 390. 1969.

3) Willett, L. B., Dunn, T. G., Brown, B. L. and Erb, R. E. Excretion of 4-^{14}C-Corticosteroids in Urine by an Ovariectomized Bovine. J. Animal Sci., 32: 119. 1971.

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Willett, L. B.

MONS 042627

Biographical Sketch

Name: Conrad, Harry R. **Birth Date:** 3 Oct. 1925

Title: Professor **Nationality:** U.S. **Sex:** Male

Birthplace: Burlington, Ky.

Education:

Institution	Location	Degree	Year
Univ. of Ky.	Lexington	B.S.	1948
The Ohio State Univ.	Columbus	M.S.	1949
The Ohio State Univ.	Columbus	Ph.D.	1952
The Univ. of Tenn. AEC Agr. Res. Program	Knoxville	Postdoc.	1955
Univ. of Calf.	Davis	Sab. leave	1961
Harvard School of Public Health	Boston	Sab. leave	1968

Honors:

Sullivan Award - 1948 - University of Kentucky (outstanding senior)
A.F.M.A. Award - 1959 - Citation by the American Dairy Science Assoc.
Borden Award - 1966 - also by the A.D.S.A. for outstanding contributions to knowledge of dairy cattle nutrition

Major Research Interest:

a. General research interest are in nutrition and physiology of ruminants.
b. Specific interests are nitrogen metabolism, factors regulating food intake and the application of tracer techniques in quantitative nutrition.

Research and Professional Experience:

Professor Conrad is currently leader of three major research projects and lecturer in Animal Energetics at the Ohio State University. He has been a member of the Graduate Faculty since 1956. Dr. Conrad has 17 years experience in ruminant research including 16 years with isotope tracer methods. Contributions include studies on radiocalcium metabolism, rumen digestion, nitrogen metabolism, blood flow, causes of anemia, radioiodine uptake in cattle, regulation of feed intake in ruminants, and essential amino acid synthesis in the rumen. He is senior author of 60 and coauthor of 51 scientific journal articles and papers. During 1961 he studied tracer methods in animal metabolism with Professors Max Kleiber and A. L. Black of the University of California. In 1968 Dr. Conrad completed a sabbatical year of study on the regulation of nutrient balance in the laboratory of Professor Jean Mayer of the Harvard School of Public Health.

Selected References:

1) Conrad, H. R., and Hansard, Sam L. Effects of Massive Doses of Vitamin D on Physiological Behavior of Calcium in Cattle. J. Applied Physiology, 10: 98. 1957.

2) Conrad, H. R., Hibbs, J. W., Pratt, A. D., and Davis, R. R. Nitrogen Metabolism in Dairy Cattle. I. The Influence of Grain and Meadow Crops Harvested as Hay, Silage, or Soilage on Efficiency of Nitrogen Utilization. J. Dairy Sci., 44: 85. 1961.

3) Conrad, H. R., Pratt, A. D., and Hibbs, J. W. Regulation of Feed Intake in Dairy Cows. I. Change in Importance of Physical and Physiological Factors with Increasing Digestibility. J. Dairy Sci., 47: 54. 1964.

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Conrad, H. R.

Biographical Sketch

Name: Waldron, Acie C. Birth Date: 4 Feb. 1930

Title: Ext. Specialist Nationality: U.S. Sex: Male
Pesticide Chemicals

Birthplace: Malad City, Idaho

Education:

Institution	Location	Degree	Year
Idaho State College	Pocatello	None	1948-50
Brigham Young Univ.	Provo, Utah	B.S.	1957
The Ohio State Univ.	Columbus	M.S.	1959
The Ohio State Univ.	Columbus	Ph.D	1961

Honors:

Sigma Xi
Gamma Sigma Delta
Who's Who in the Midwest

Major Research Interest:

Pesticide residues in agricultural crops and livestock, in soils, and in water; and the safe use of pesticides.

Research and Professional Experience:

1. Extension Specialist, Pesticide Chemicals, Ohio Cooperative Extension Service, 1966-present. Major responsibility - supervision of Pesticides Analytical Laboratory concerned with determination of pesticide residues in agricultural crops, livestock, soils, and water. Pesticide Coordinator - dissemination of information on pesticide regulations and registrations, pesticide safety, environmental concerns with pesticides and all similar information. Promotion of the pesticide safety program.

2. Method Development Chemist, Agricultural Division, American Cyanamid Co., Princeton, New Jersey. 1961-1966. Concerned with the development of analytical methods for the determination of pesticide residues in plant and animal tissues and in formulations.

3. Research Assistant, Ohio State Experiment Station, O.S.U., Columbus, Ohio 1957-1961. In conjunction with graduate work. Studied the extraction, separation, and identification of organic nitrogen and phosphorus compounds in soil

organic matter and the composition of the fluorescent fraction of soil organic matter.

4. Publications Editor, U.S. Army Chemical Corp. Commissioned Officer duties involved writing and revising Chemical Corps field manuals and sub-courses.

Selected References:

1) Waldron, A. C. and Mortensen, J. L. Soil Nitrogen Complexes: III. Distribution and Identification of Nitrogenous Constituents in Electrophoretic Separates. Soil Sci. 93: 286-293. 1962.

2) Waldron, A. C., Pasarela, N.R., Woolford, M. H. and Ware, J. H. Improved Colorimetric Determination of Phorate Residues in Plant Tissues. J. Agr. & Food Chem. 11: 241-244. 1963.

3) Pasarela, N. R. and Waldron, A. C. Fluorometric Method for Microgram Quantities of Tehylenediamine Residues in Milk. J. Agr. & Food Chem. 15: 221-226. 1967.

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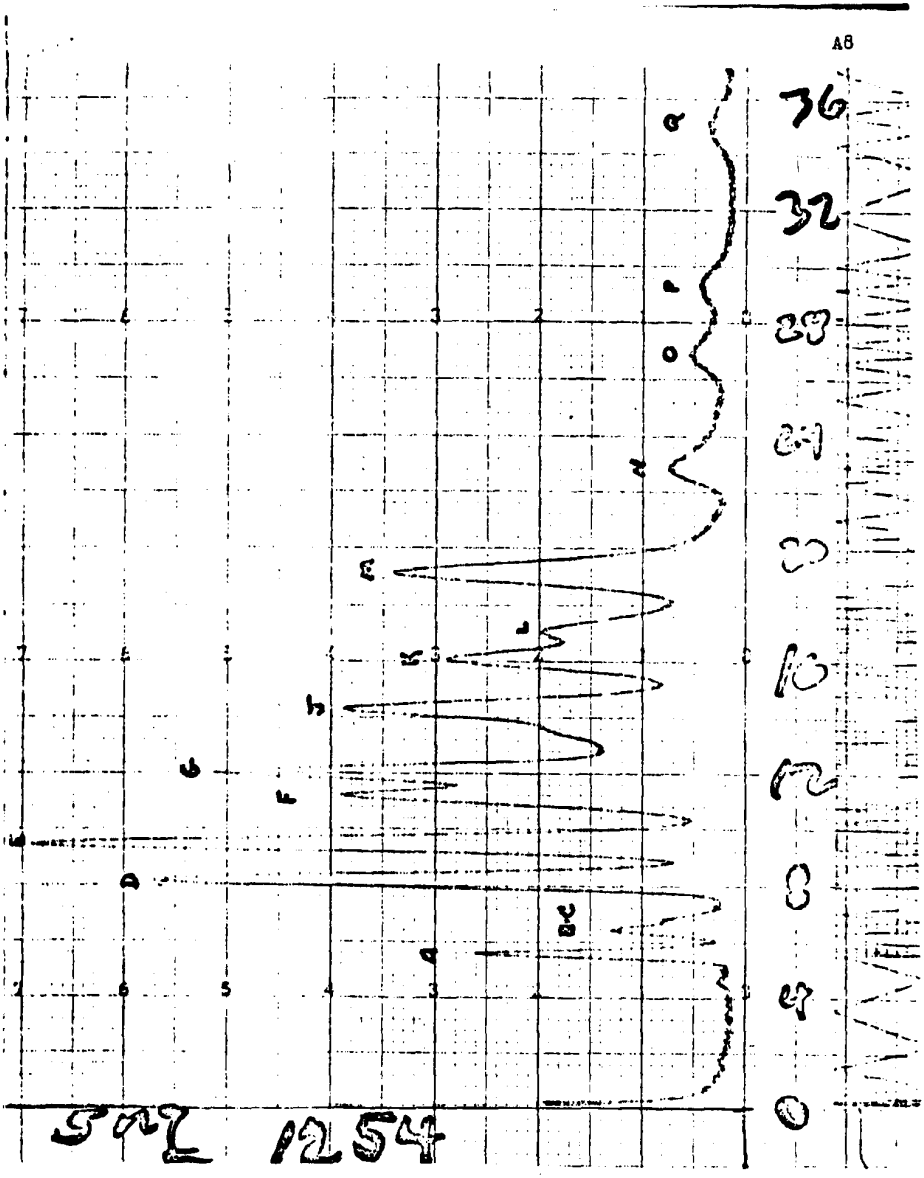

MONS 042632