Nitrosamines in Agricultural and Home-Use Pesticides

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Over 90 technical and commercial pesticide formulations used in agriculture, hospitals, and homes were screened for presence of *N*-nitroso compounds. Twenty-five were found to contain nitrosamines at or above 1 ppm. The methodology and instrumentation used are discussed in detail. In addition, the formation of *N*-nitrosodimethylamine in an amine salt herbicide formulation after addition of sodium nitrite is demonstrated.

Recent reports have indicated a correlation between the incidence of cancer in test animals and the ingestion or inhalation of nitrosamine compounds. In 1956 Magee and Barnes demonstrated that feeding *N*-nitrosodimethylamine (DMNA) to rats produced malignant hepatic tumors. The possible role of nitrosamines in tobacco smoke and lung cancer was suggested by Lijinsky and Epstein (1970).

In 1976 Fine reported finding *N*-nitroso compounds in pesticide formulations which are used extensively in agriculture. Some of these products, such as trifluralin, were reported to contain as much as 154 parts per million (ppm) of dipropylnitrosamine. Such reported levels of nitrosamines were of immediate concern to EPA's Office of Pesticide Programs. As a result, EPA scientists initiated a survey to determine the extent of nitrosamine contamination in technical and formulated products used both in agriculture and in or around homes. The survey samples were selected on the basis of the three most probable routes of *N*-nitroso contamination: (1) formation in the manufacturing process, (2) formation during storage, and (3) contamination of amines used in the manufacturing process.

METHODOLOGY

N-Nitroso compounds were designated as volatile or nonvolatile. Those which could be successfully chromatographed on a gas–liquid chromatograph (GLC) were considered volatile and those which could not were labeled nonvolatile. Three analytical methods were used for the analysis of *N*-nitroso volatiles. These were GLC with a thermal energy analyzer detector (TEA), GLC with a Hall electrolytic conductivity detector, and a high-pressure liquid chromatograph (LC) with both an ultraviolet (UV) and TEA detector. For the nonvolatiles LC–UV and LC–TEA were used. Samples were divided into volatile and nonvolatile groups depending on the nature of the nitrosamine suspected. The volatiles were run on the GLC–TEA. Positive responses were confirmed by GLC–Hall or LC–UV–TEA where possible. Nonvolatile samples were run on LC–TEA and confirmed by LC–UV where possible. A discussion of the theory and operation of the TEA can be found in the literature (Fine and Rufeh, 1974). However, the authors would like to offer a few suggestions for efficient use of the TEA.

When using the TEA it is absolutely necessary to clean the glass cold traps used for LC with sulfuric dichromate cleaning solution before reusing. Failure to do this results in a high background which substantially lowers detection sensitivity. Likewise, observation of decreased sensitivity in the GLC mode usually indicates the need to clean the metal trap with acetone.

The authors also recommend a periodic check of the ozonator to determine if cleaning is needed. If the ozonator has a brown or grey-black appearance, it is necessary to buff it with emery cloth until a shiny metallic surface is obtained. Such maintenance will insure maximum sensitivity of detection.

An additional precaution is to refrain from introducing too much LC solvent into the pyrolysis furnace. Failure to do so results in coating the pyrolysis chamber, which prevents complete pyrolysis of the sample eluant. This in turn will also give a high background which prohibits any further analysis and requires either sending the furnace to the manufacturer for cleaning or replacing. The authors recommend that for LC–TEA analysis a retention time for the nonvolatile *N*-nitroso compound of interest be established using a standard material and the LC–UV system alone. After this is done, one can vent most of the LC mobile solvent before it reaches the TEA pyrolysis chamber, and within 30 s to a minute of elution time, introduce the solvent into the TEA pyrolysis chamber. One other precaution is to refrain from direct injection of formulations into the pyrolysis chamber. The experience of the authors indicates that such injections will coat the chamber and give an unacceptable base line.

Finally, it is also necessary to cover the traps *completely* with dry ice and methanol to insure a relatively noise-free base line. If these precautions are followed, the TEA can be used to its maximum efficiency.

EXPERIMENTAL SECTION

Because the laboratory was required to screen a large number of samples within a short amount of time, a screening level of 1 ppm was chosen. Other modes of detection, such as GLC–flame ionization, are not sensitive enough for our analysis. Analysis of nonvolatile *N*-nitroso compounds requires LC–TEA and/or LC–UV, the former having a sensitivity of an order of magnitude less than GLC–TEA; however, LC–UV would have a sensitivity of less than 3 ppm.

Operating parameters and LC solvent systems for the analysis of some samples are shown in Figures 1 and 2. All solvents were residue analysis grade by Burdick and Jackson, J. T. Baker, or MCB except the absolute ethanol which was reagent grade by U.S. Industrial Chemical Co. None of the solvents were purified further before use.

Some nitrosamine standards were synthesized and furnished by Larry Keefer of the National Cancer Institute, National Institutes of Health, Bethesda, MD. Other standards were furnished by Dow Chemical Co., Midland, MI.; American Cyanamid Co., Princeton, NJ; Ciba Geigy

U.S. Environmental Protection Agency, Office of Pesticide Programs, Benefits and Field Studies Division, Chemical and Biological Investigations Branch, Beltsville, Maryland 20705.

Instrument Operating Parameters

	Column	T°C	Flow Rate
GLC-TEA	14 ft-1/8" 10% Carbowax-20M & 0.05% KOH on Chromosorb WHP-80/100	175	30 ml/min
GLC-HALL DETECTOR	6 ft-1/4" 3% Carbowax-20M on Chromosorb W-80/100	120	30 ml/min
HPLC-TEA HPLC-UV (254 nm)	2 – 3.9 mm ID x 30 cm μ Porasil connected in Tandem		1.5 ml/min

HPLC Solvent Systems

HPLC-UV	
Volatile Nitrosamines	15% Isopropanol in Iso-Octane
Non-Volatile Nitrosamines in:	
Triazine Herbicides Prowl Butralin	3% Dimethoxyethane in Iso-Octane plus 0.02% 75/25 (Isopropanol-Water)
Diethanolamine Salts	50% Dimethoxyethane in Iso-Octane plus 0.02% 75/25 (Isopropanol-Water)
HPLC-TEA	
Volatile Nitrosamines Non-Volatile Nitrosamines	10% Acetone in Iso-Octane
Diethanolamine Salts	40/60 Acetone-Iso-Octane

Figure 1. Typical instrument operating parameters for GLC and LC analysis for nitrosamines.

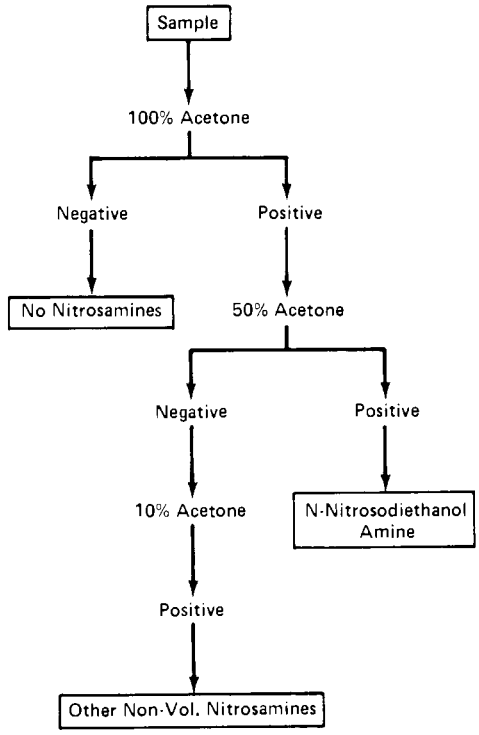
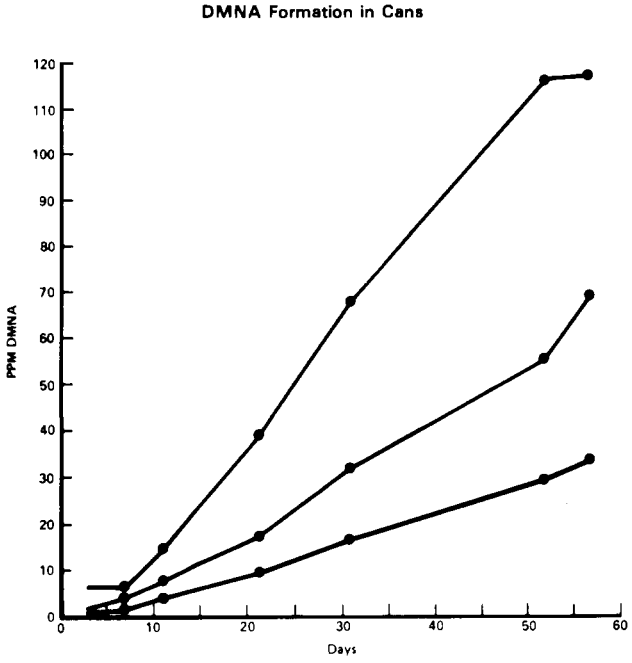


Figure 2. Procedure for screening and identifying nonvolatile nitrosamines on LC–TEA showing different solvent systems used.

Corp., Greensboro, NC; Amchem Products Inc., Ambler, PA; and William Lijinsky, Director, Chemical Carcinogenesis Program, Frederick Cancer Research Center, Frederick, MD. All standards were used as received from the source.

The GLC Model 560 and Hall detector Model 700 were manufactured by Tracor Instruments. High-pressure liquid chromatograph pump, solvent programmer Model



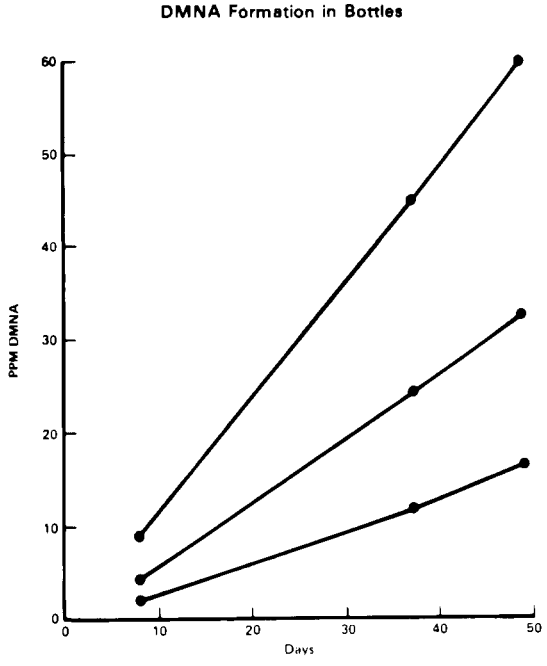


Figure 3. Formation of dimethylnitrosamine in a formulation of dimethylamine salt of 2,4-D stored in metal cans and glass bottles after addition of sodium nitrite. The three curves correspond to addition of 0.1% (lower curve), 0.2% (middle curve), and 0.5% (upper curve) sodium nitrite.

660, and UV absorbance detector Model 440 were manufactured by Waters Associates. The thermal energy analyzer (TEA) Model 502 and GLC Model 661 were manufactured by Thermo Electron Corporation, Waltham, MA.

All samples were sent directly by the manufacturer or collected by Consumer Safety Officers of the Environmental Protection Agency.

One-half gram of sample was placed in a vial to which was added 10 mL of ethanol or hexane. After settling, 10 μL of the clear supernatant liquid was injected into the GLC or LC interfaced with the TEA.

In the analysis of some of the dinitrobutyl aniline

Table I. Triazine Herbicides Screened for Nitrosoamine Contamination[a]

sample	ingredient
N-1	hexahydro-1,3,5-triethyl-*s*-triazine
N-2	4,6-dichloro-*N*-(2-chlorophenyl)-1,3,5-triazin-2-amine
N-3	metribuzin
N-4	hexahydro-1,3,5-tris(2-hydroxypropyl)-*s*-triazine
N-5	2-[(4-chloro-6-(ethylamino)-*s*-triazin-2-yl) amino]-2-methylpropionitrile
N-9	2-chloro-4-cyclopropylamino-6-isopropylamino-*s*-triazine
N-10	2-(*tert*-butylamino)-4-chloro-6-(ethylamino)-*s*-triazine
N-11	2-chloro-4,6-bis(ethylamino)-1,3,5-triazine
N-12	2-chloro-4,6-bis(isopropylamino)-1,3,5-triazine
N-13	2-(ethylthio)-4,6-bis-(isopropylamino)-1,3,5-triazine
N-15	2-methoxy-4,6-bis(isopropylamino)-1,3,5-triazine
N-16	2-methylthio-4,6-bis(isopropylamino)-1,3,5-triazine
N-17	2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine
N-18	2-ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine
N-26	same as N-17
N-27	same as N-17
N-48	same as N-17
N-49	same as N-17
N-50	same as N-17
N-62	same as N-17
N-73	same as N-5

[a] No nitrosamine was detected over 1 ppm.

products, occasional interference prevented resolution of the nitrosamine peak. This interference was eliminated by column chromatography using an alumina-packed glass column. The sample was dissolved in benzene and transferred to the alumina column. The interfering materials were washed off with hexane, and the nitrosamine was eluted with benzene. Interferences that appeared on LC–UV chromatograms were eliminated by changing the polarity of the mobile solvent.

A sample of a dimethylamine salt of 2,4-D formulation containing neither nitrite nor dimethylnitrosamine was divided into eight subsamples. Three of the samples were spiked with 0.1, 0.2, and 0.5% sodium nitrite, and one was used as a blank. These four were placed in metal cans. Duplicate samples were placed in glass bottles. Each sample was analyzed for DMNA at intervals of several days. The rapid formation of dimethylnitrosamine is shown in Figure 3. Note that DMNA was formed more rapidly in cans than in bottles. This may be due to a catalytic effect of the metal cans. The blank of each set showed no formation of nitrosamine over the entire period of the study.

RESULTS AND DISCUSSION

The analytical findings are shown in Tables I–V. The tables of results are listed for each of the three routes for the formation of *N*-nitroso compounds in agricultural and home use products. If the laboratory did not have a standard of the nitrosamine which would be logically expected in a specific product, then it did not analyze for that nitrosamine.

Table II. Dinitroaniline and Similar Herbicides Screened for Nitrosamine Contamination[a]

sample	ingredient	GLC-TEA, ppm	LC-TEA, ppm	LC-UV, ppm	GLC-Hall, ppm
N-20	2,6-dinitro-*N*,*N*-dipropyl-4-trifluoromethylaniline	121 DPNA[b]			150 DPNA
N-21	3,5-dinitro-N^4,N^4-dipropylsulfanilamide				
N-22	4-isopropyl-2,6-dinitro-*N*,*N*-dipropylaniline	54 DPNA			39 DPNA
N-32	2,6-dinitro-*N*,*N*-dipropyl-4-trifluoromethylaniline	11 DPNA			13 DPNA
N-37	4-(1,1-dimethylethyl)-*N*-(1-methylpropyl)-2,6-dinitrobenzamine			74 nitrosamine of parent compd	
N-38	*N*-butyl-*N*-ethyl-2,6-dinitro-4-trifluoromethylaniline	38 BENA[c]			28 BENA
N-40	3,5-dinitro-N^4,N^4-dipropylsulfanilamide	sub 1, 1.5 DPNA			
		sub 2, neg	neg[f]		
N-51	N^3,N^3-diethyl-2,4-dinitro-6-trifluoromethyl-1,3-phenylenediamine	sub 1, 153 DENA[d]	146		
		sub 2, 100 DENA	85		
N-63	3,5-dinitro-N^4,N^4-dipropylsulfanilamide				
N-64	*N*-butyl-*N*-ethyl-2,6-dinitro-4-trifluoromethylaniline	8 BENA	3		
N-65	*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine (technical)		102 nitrosamine of parent compd	104 nitrosamine of parent compd	
N-66	*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine (anal. std)		neg		
N-75	2,6-dinitro-*N*,*N*-dipropyl-4-trifluoromethylaniline	16 DPNA	present		
N-80	4-isopropyl-2,6-dinitro-*N*,*N*-dipropylaniline	9 DPNA	present		
N-87	2,6-dinitro-*N*,*N*-dipropyl-4-trifluoromethylaniline	6 DPNA			
N-91	*N*-(cyclopropylmethyl)-α,α,α-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine	4 CMPNA[e]			

[a] A blank in the table means the sample was not analyzed by that method. [b] *N*-Nitrosodi-*n*-propylnitrosamine. [c] *N*-Nitroso-*n*-butylethylnitrosamine. [d] Diethylnitrosamine. [e] *N*-Nitrosocyclopropylmethyl-*n*-propylnitrosamine. [f] Less than 1 ppm.

Table III. Amine Salt Herbicides Screened for Nitrosamine Contamination

sample	ingredient	GLC-TEA, ppm	LC-TEA, ppm	LC-UV, ppm	GC-Hall ppm
N-6	dimethylamine salt of 2,4-D and 2,4,5-trichlorophenoxypropionic acid	neg[a]	c		
N-8	dimethylamine salt of 2,4-D	6 DMNA[b]			
N-23	dimethylamine salt of 2-methyl-4-chlorophenoxyacetic acid	neg			
N-24	dimethylamine salt of 2-(2-methyl-4-chlorophenoxy)propionic acid	2 DMNA	neg		
N-33	dimethylamine salt of 2,3,6-trichlorobenzoic acid	2 DMNA			
N-34	dimethylamine salt of 2,3,6-trichlorobenzoic acid	253 DMNA			250 DMNA
N-35	diethanolamine salt of 2,4-D	neg	neg		
N-36	dimethylamine salt of 2,4-D	neg			
N-42	diethanolamine salt of 6-hydroxy-3(2*H*)-pyridazinone		neg	neg	
N-43	dimethylamine salt of 2,4-D	neg			
N-44	dimethylamine salt of 2-methyl-4-chlorophenoxyacetic acid	neg			
N-45	dimethylamine salt of 4-(2,4-dichlorophenoxy)butyric acid	2.5 DMNA	6		
N-46	dimethylamine salt of 2,4-D	neg			
N-47	diethanolamine salt of 2-(2-methyl-4-chlorophenoxy)propionic acid diethanolamine salt of 2,4-D		neg		
N-52	diethanolamine salt of 2,4-D		neg		
N-54	dimethylamine salt of 2,3,6-trichlorophenylacetic acid	18 DMNA	19, 24		
N-55	morpholine salt of 2,4-D	neg			
N-56	dimethylamine salt of 2,4-D	neg			
N-57	dimethylamine salt of 2-methyl-4-chlorophenoxyacetic acid	neg			
N-58	diethanolamine salt of 2,4-D diethanolamine salt of 2-(2-methyl-4-chlorophenoxy)propionic acid		neg		
N-59	diethanolamine salt of 2,4-D		neg		
N-61	diethanolamine salt of 6-hydroxy-3(2*H*)-pyridazinone		neg		
N-71	dimethylamine salt of 2,4-D dimethylamine salt of 2-(2,4,5-trichlorophenoxy)propionic acid	neg			
N-72	dimethylamine salt of 2,4-D	neg			
N-78	dimethylamine salt of 2-(2-methyl-4-chlorophenoxy)propionic acid dimethylamine salt of 2,4-D	1 DMNA	neg		
N-79	dimethylamine salt of 2,4-D dimethylamine salt of 2-(2-methyl-4-chlorophenoxy)propionic acid	neg			
N-82	dimethylamine salt of 2,3,6-trichlorobenzoic acid	neg			
N-83	diethanolamine salt of 3-trifluoromethylsulfonamido-*p*-acetotoluidide	neg			

[a] Less than 1 ppm. [b] Dimethylnitrosamine. [c] A blank indicates that the sample was not run by that method.

Table IV. Alkyl Amines Used in Manufacturing Screened for Nitrosamine Contamination

sample	ingredient	GLC-TEA, ppm	LC-TEA, ppm	LC-UV, ppm	GLC-Hall, ppm
N-28	dimethylamine	34 DMNA	b	26 DMNA	
N-31	dimethylamine	28 DMNA		29 DMNA	
N-67	triethanolamine		neg[a]		
N-69	diethanolamine		neg		
N-85	dimethylamine	4 DMNA	6 DMNA		

[a] Less than 1 ppm. [b] A blank indicates that the sample was not analyzed by that method.

With the exception of atrazine, none of the triazine herbicides were analyzed for nonvolatile nitrosamines. However, the analyses did not indicate the presence of any volatile nitrosamine in any of these products.

The finding of *N*-nitroso compounds in dinitroaniline products probably results from a reaction of residual HNO_2 that is left from the nitration of the chlorobenzene and the excess secondary amine used in the amination step.

N-Nitroso compounds in formulations of amine salts of phenoxy herbicides probably result from the reaction of nitrite, a corrosion inhibitor, and the corresponding secondary amine used to form the amine salt. This possibility was demonstrated in the nitrite study previously discussed.

Results of the analyses of other herbicide and pesticide materials are shown in Table V. The presence of a relatively large quantity of *N*-nitrosodiethanolamine in sample N-68 cannot be explained at this time. Analysis of the amine used to manufacture this compound did not indicate the presence of the corresponding nitrosamine. The nitrosamine presence may be a result of a synthesis step in the making of the 2-*sec*-butyl-4,6-dinitrophenol. In those samples that were analyzed by more than one method the agreement was fairly good.

CONCLUSIONS

Of the 91 pesticides and starting materials screened, 25 contained nitrosamines at or above 1 ppm; 14 of these were the dinitroaniline formulations, 7 were amine salts, 3 were amines used in the manufacturing process, and 1 was the sample containing *N*-nitrosodiethanolamine.

The results indicate that the higher levels of *N*-nitrosamines are primarily found in substituted amine, dinitroaniline, and amine salt formulations whereas the triazine compounds were free from nitrosamine contamination. It is also evident that the presence of nitrite in amine salt formulations or products containing secondary amines will cause formation of *N*-nitrosamines, regardless of whether they are stored in glass or metal containers.

EPA neither endorses nor recommends any manufacturer or products mentioned in this paper. Brand names were given only as a source of supply.

SAFETY

Nitrosamines are potent animal carcinogens and are suspected human carcinogens. All work with pure standards should be done in a glovebox which is vented to a hood. The authors recommend using protective clothing when handling diluted standards and samples. If possible, a separate laboratory and equipment should be

Table V. Miscellaneous Herbicides Screened for Nitrosamine Contamination; Other Pesticides Screened for Nitrosamine Contamination

sample	ingredient	GLC-TEA, ppm	LC-TEA, ppm	LC-UV, ppm	GLC-Hall, ppm
	Miscellaneous Herbicides				
N-19	3,6-dichloro-*o*-anisic acid	neg[b]	*d*		
N-68[a]	diethanolamine salt of 2-*sec*-butyl-4,6-dinitrophenol		233 DELNA[c]	217 DELNA	
N-70	2-*sec*-butyl-4,6-dinitrophenol	neg			
N-81	3-(3,4-dichlorophenyl)-1,1-dimethylurea	neg			
N-86	3-(3,4-dichlorophenyl)-1,1-dimethylurea	neg			
N-88	2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate	neg			
N-89	same as N-88	neg			
	Other Pesticides				
N-41	bis(dimethylthio)carbamoyl) disulfide	neg			
N-60	diphenylamine	neg			
N-76	sodium [4-(dimethylamino)phenyl]diazene sulfonate	neg			
N-77	same as N-76	neg			
N-84	bis(dimethylthiocarbamoyl) disulfide	neg			
N-7	2,3,5-triiodobenzoic acid	neg			

[a] Presumed to be diethanolamine salt. [b] Less than 1 ppm. [c] Diethanolnitrosamine. [d] A blank indicates that the sample was not analyzed by that method.

used when working with nitrosamines. All trash from the laboratory should be disposed of separately if possible.

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COMMUNICATIONS

Protein Quality Evaluation of Spent Hops

Protein quality of hops, from which the resin had been hexane extracted, was evaluated. Total protein content of the spent hops was 22.43%. A complete amino acid profile of spent hops was determined and evaluated with regard to human requirements. Calculation of a computerized protein efficiency ratio (C-PER) revealed a very low C-PER value (0.11). The low C-PER value was attributed to the low in vitro protein digestibility, 64.52%, as well as the low levels of sulfur amino acids and lysine. By comparing the amino acid profiles of the whole hop plant and spent hops, it was evident that the extraction process significantly reduces the protein efficiency ratio of hop proteins. Feed manufacturers may find the amino acid profile of spent hops useful for formulating rations as it has not been published elsewhere.

Spent hops are a byproduct of the hop-extraction process. U.S. hop production in 1977 totaled 54.8 million pounds. More than 16 million pounds were used for extract production. Only 30% of the hop is extractable as a resin for use in the brewing of beer. Thus, there was in 1977 an excess of 12 million pounds of vegetative discard or spent hops.

Early research of this byproduct was directed toward its use as a feed for sheep (Kellner, 1879; Weiske et al., 1879). Davies and Sullivan (1927) were the first to report animal feeding trials on spent hops in the United States. They observed that spent hops were not eaten readily by sheep and could only be included in a ration in an amount equal to about one-seventh of the dry weight of the total ration. All of these early experiments were conducted with low numbers of sheep that were fed for very limited periods of time. Furthermore, the spent hops used in these early studies were extracted with water rather than with hexane. Nevertheless, the observation that spent hops are not of as high a quality as conventional forages remains accurate.

The use of spent hops as an animal feed was not reported again until 1974, when Heinemann and Dyer conducted feeding studies with cattle. In a steer feeding trial they found that spent hops satisfactorily replaced one-third of the alfalfa cubes. These workers found that spent hops contained approximately 20.6% protein, a relatively high protein content. In an effort to develop a high value byproduct, the quality of the protein for man

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