DNT's - Deer Study.

December 23, 1991

Commander's Representative

SUBJECT: Study of Dinitrotoluene in Deer Tissue at Badger Army Ammunition Plant

Ms. Meg Ziarnik
Wisconsin Dept. of Health & Social Services
Division of Health
1 West Wilson St., P.O. Box 309
Madison, WI 53701-0309

Dear Ms. Ziarnik:

Subject report is attached for your information.

Please contact me at (608)356-5525 if there are any questions.

Sincerely,

Original signed by

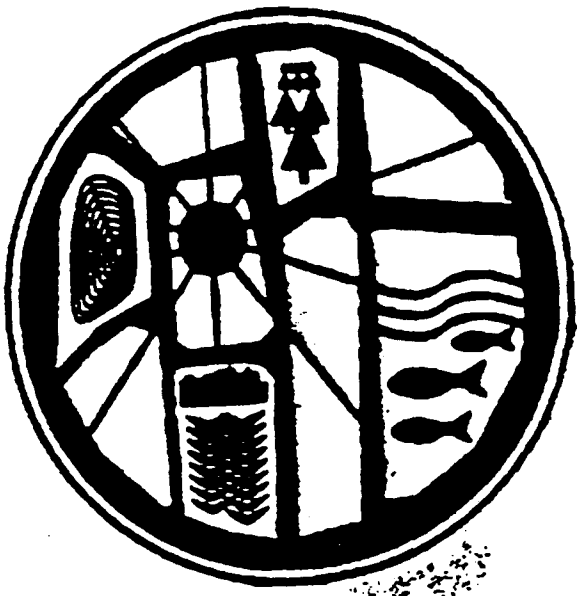
David C. Fordham
Commander's Representative

Attachment

Copy Furnished:
Sauk County Public Health, ATTN: B. Wickus
WDNR, ATTN: M. Netzer
USEPA, ATTN: R. Egan
Cdr, AMCCOM
ATTN: AMSMC-SG
AMSMC-ISE
AMSMC-ISF-R
Cdr, USATHAMA
ATTN: CETHA-IRA
SWAB

Copy Capt. Jones, Joliet AAP 1/15/92 mc

ORNL/M-1765



DINITROTOLUENE IN DEER TISSUES

FINAL REPORT

Lee R. Shugart
Environmental Sciences Division
Oak Ridge National Laboratory

ESD Publication No. 3802

September 30, 1991

ORNL/M-1765

DINITROTOLUENE IN DEER TISSUES

Final Report

L. R. Shugart

Environmental Science Division
Publication No. 3802

Date Published - - September 30, 1991

Prepared for

Olin Corporation
Winchester Group Ammunition Operations
Badger Army Ammunition Plant
Baraboo, Wisconsin 53913

Prepared by the

Environmental Sciences Division
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831-6036
managed by
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for the
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ORNL/M-1765

Contract No.:

DOE Project No. ERD-90-1001

Contract Title:

DINITROTOLUENE IN DEER TISSUES

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Report Date:

September 30, 1990

Type of Report:

Final Report

Contracting Officer's Technical Representative:

George Shalabi
Olin Corporation
Winchester Group Ammunition Operations
Badger Army Ammunition Plant
Baraboo, Wisconsin 53913

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ABBREVIATIONS

BAAP	Badger Army Ammunition Plant, Baraboo, Wisconsin
CL	Criterion Level
DL	Detection Level
1,3-DNB	1,3-dinitrobenzene (Figure 2)
2,4-DNT	2,4-dinitrotoluene (Figure 2)
2,6-DNT	2,6-dinitrotoluene (Figure 2)
HPLC	High Performance Liquid Chromatography
ORNL	Oak Ridge National Laboratory
s.e.m.	standard error of the mean

Units of Measure

C	centigrade
g	gram
mg	milligram (g X 10^{-3})
kg	kilogram (g x 10^{3})
ng	nanogram (g X 10^{-9})
min	minute
hr	hour
mL	milliliter (L X 10^{-3})
µL	microliter (L X 10^{-6})
ppm	part per million
min	minute
mm	millimeter (m X 10^{-3})
nm	nanometer (m X 10^{-9})

1. PURPOSE AND SCOPE OF PROJECT

Badger Army Ammunition Plant (BAAP), Baraboo, Wisconsin, has within a security-fenced area, a herd of whitetail deer. The U.S. Army and the State of Wisconsin, Department of Health and Social Services have determined that approximately 20 of the deer be harvested and tissue samples thus collected be analyzed for 2,4- and 2,6-dinitrotoluene (2,4- and 2,6-DNT) by high pressure liquid chromatography (HPLC) to a sensitivity of 0.1 part per million (ppm). The HPLC analyses will be done at the Oak Ridge National Laboratory (ORNL) following protocol used previously for similar work for other government sites. ORNL shall instruct Olin relative to the quantity and type of tissue required, storage and shipment requirements, and other information to ensure that all protocol and chain of custody requirements are clear. A final report will be made to Olin Corporation upon completion of the HPLC analyses.

2. WORK PERFORMED

2.1 COLLECTION OF TISSUE

Tissue samples were obtained from deer hunted on the BAAP reservation. The total population of deer at the BAAP site was estimated to be approximately 430 (see Appendix A). Collection of appropriate samples was under the supervision of BAAP personnel, who forwarded them to ORNL for analysis. Thirty-six individual samples weighing approximately 100 g each of liver, muscle, and heart tissue from twelve individual deer were received at ORNL on January 10, 1991. These samples were stored at -20°C awaiting analysis for 2,4-DNT, and 2,6-DNT. In addition, control samples of deer liver, muscle, and heart tissue were obtained from non-munition-contaminated animals. These animals were collected on the U.S. Department of Energy Oak Ridge Reservation or at the Catoosa Wildlife Management Area in eastern Tennessee. This collection was under the supervision of personnel from the State of Tennessee Wildlife Resources Agency, and all tissue collected were archived at -20 °C at ORNL.

2.2 METHODS OF ANALYSIS

2.2.1 Extraction of Tissue

Each sample of deer tissue obtained from the BAAP site was analyzed in duplicate, as were similar tissues from several deer taken from the U.S. Department of Energy Reservation and the Catoosa Wildlife Management Area. Approximately 2 g of tissue sample was used for each analysis, and 200 ng of 1,3-dinitrobenzene (1,3-DNB) was added as an internal standard.

The protocol used to extract 2,4-DNT and 2,6-DNT from animal tissues for subsequent HPLC analysis was a modification of that used by Shugart et al. (1991) for the extraction of these and other munitions-like compounds from tissues of various animals including deer. The procedure included the following steps:

1. In the presence of liquid nitrogen, grind tissue in mortar and pestle, spike with 200 ng of 1,3-DNB (and the two isomers of DNT when required) and transfer sample to a glass vial.

2. Add 6 mL of acetonitrile and mix well to disperse tissue. After 1 hr at room temperature, pass contents through a glass fiber filter and collect filtrate. Wash vial and filter with an additional 6 mL of acetonitrile, and pool filtrates.

3. Using nitrogen gas, evaporate the filtrate to a volume of approximately 1 mL, and add 10 mL of water. Mix thoroughly, and pass the solution through a SepPak, C18 solid-phase extraction column (Waters). Wash column with 2 mL of water, and elute the sample from the column with 1 mL of ethylacetate. Collect the eluate.

4. Remove 250 µL from the upper, organic phase of the eluate and evaporate to dryness with nitrogen gas. Dissolve the sample in 50 µL of ethylacetate for HPLC analysis.

2.2.2 HPLC

Analysis was by reverse-phase column chromatography on a Brownlee Lab. column (Spheri-5, RP-18, 5 micron, 220 X 4.6 mm) maintained at room temperature. Sample size was 20 µL, and isocratic elution was performed with 50% methanol/water at a flow

rate of 1 mL/min. Detection was at 254 nm with an Altex UV monitor usually set at a sensitivity range of 0.04 (i.e., total range on chart was 0.04 absorbance units at 254 nm). The chromatogram generated by each sample was recorded at a chart speed of 2 mm/min, and each was examined individually to determine recovery of the internal standard (1,3-DNB) and the presence of either 2,4- or 2,6-DNT.

Authentic standards of 1,3-DNB, 2,4-DNT, and 2,6-DNT at a concentration of 1 mg/mL in acetonitrile were obtained from Dr. John Caton of the Analytical Chemistry Division (ORNL). Standards were stored at 4°C in a sealed brown-glass vial.

2.2.3 Statistical Analysis

A statistical design was formulated to determine if a chemical is likely to be present in the tissues of an animal population at or above a designated criterion level (CL) when only a portion of that population has been sampled has been formulated (Beauchamp et al. 1991, included as Appendix B to this report). The statistical design was used to analyze the data generated in this work.

3. RESULTS

3.1 DEVELOPMENT OF HPLC PROCEDURE

Previous work at ORNL on the separation and identification of munition-like chemicals demonstrated that the two isomers of DNT (2,4- and 2,6-) could be easily analyzed by HPLC (Shugart et al. 1991). A simplified HPLC procedure (as detailed in Sect. 2) was adopted for this project.

A typical chromatogram generated by the HPLC procedure for the three authentic compounds of interest (1,3-DNB, 2,4- and 2,6-DNT) is shown in Fig. 1. The sensitivity of the monitor was set to 0.04, and the injected sample contained 200 ng of each compound. For quality control and to check the performance of the HPLC system, a separate HPLC chromatogram was obtained using the authentic compounds each time animal samples for this project were analyzed (i.e., the peak height and elution position of each authentic compound were verified). The following data on the mean peak heights ($\pm$ s.e.m.) of each of the authentic compounds taken from 17 different HPLC chromatograms produced during this project indicate that the HPLC procedure performed satisfactorily: 1,3-DNB (85 $\pm$1.7), 2,6-DNT (31 $\pm$1.0), and 2,4-DNT (65 $\pm$2.2).

Data on the linearity of response of the monitor utilized with the HPLC system and the lower limit of detection of the three authentic compounds are graphically presented in Fig. 2. (Each data point represents the average of two chromatographic runs.)

Figure 1. HPLC Chromatogram of 1,3-dintrobenzene; 2,4-dinitrotoluene; and 2,6-dinitrotoluene

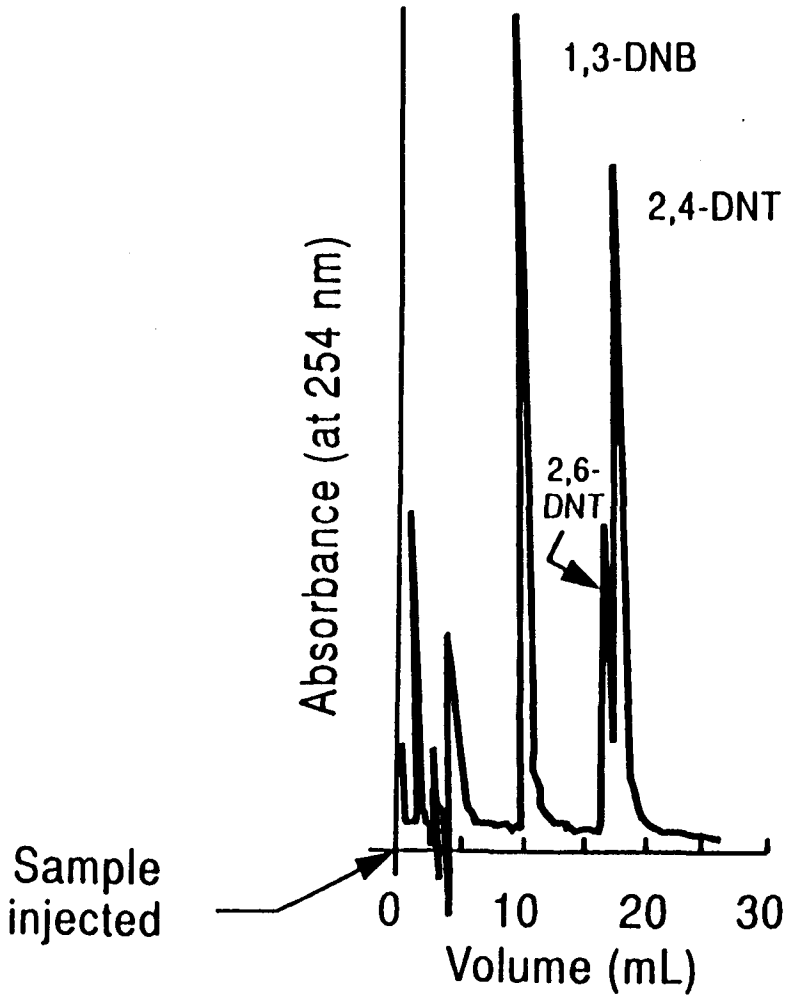
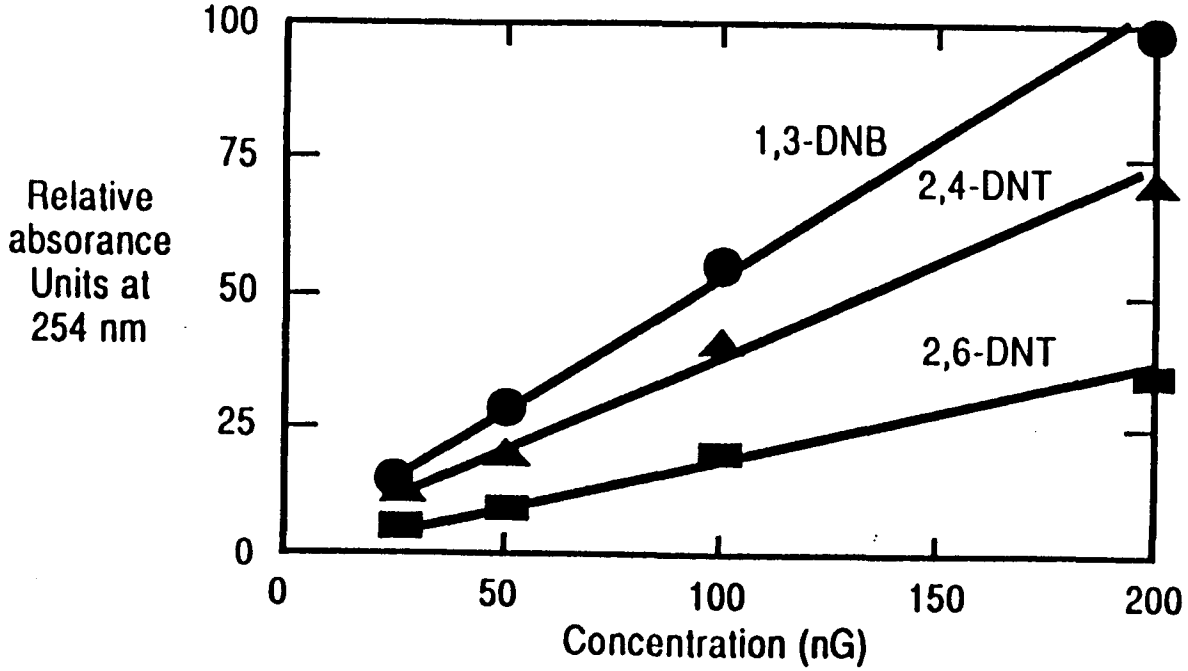
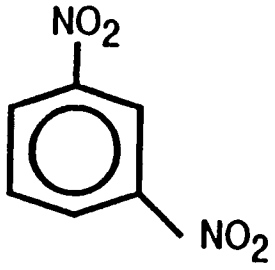
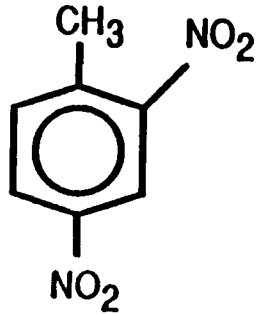


Figure 2. Peak Heights (as absorbance units at 254 nm) For Varying Concentrations of 1,3-dinitrobenzene; 2,4-dinitrotoluene; and 2,6-dinitrotoluene

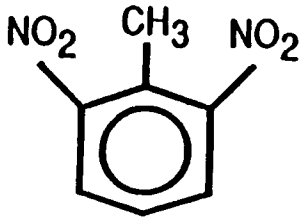




1,3 - Dinitrobenzene



2,4 - Dinitrotoluene



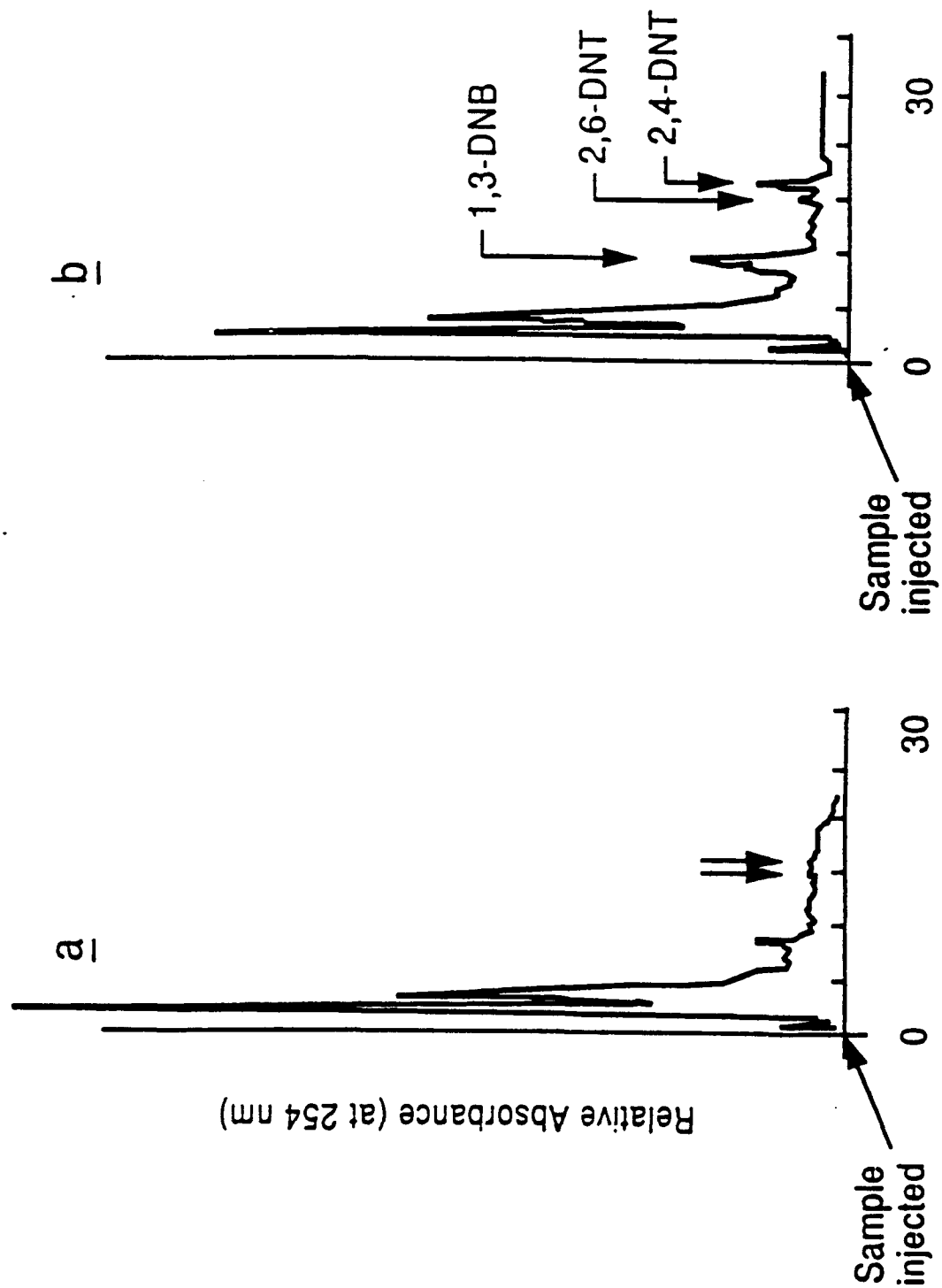
2,6 Dinitrotoluene

3.2 EXTRACTION OF ANIMAL TISSUES

It is stated in Sect. 1 of this report that the deer tissue samples obtained from the BAAP site would be analyzed for the presence of 2,4- and 2,6-DNT by HPLC to a sensitivity of 0.1 ppm. This means that the methods employed (tissue extraction followed by HPLC analysis) would detect, as a lower limit, the presence of 100 ng of either compound in 1 g of deer tissue. (Note: in Sect. 3.4, and Sect. 4, this limit is referred to as the Detection Limit.) Because each sample of deer tissue taken for analysis weighed approximately 2 g, the presence in these samples of either compound at a concentration of 0.1 ppm results in 200 ng being available for analysis. Because the overall extraction procedure (see Sect. 2.2.1) results in 1/6th of any compound present being injected in a single HPLC run the total amount of any compound available for HPLC analysis would be 33.3 ng (assuming 100% recovery of each compound through the extraction procedure). (For point of reference it should be noted that in step 4 of the protocol used to extract compounds from tissue, only 0.600 mL of the ethylacetate used to wash the SepPac C18 column is actually recovered.) Recovery data (percentage compound recovered $\pm$s.e.m.) for five different 2-g samples of deer liver tissue, each spiked with 200 ng of the authentic compounds, was 1,3-DNB (87.2% $\pm$3.5), 2,6-DNT (90.2% $\pm$4.0), and 2,4-DNT (92.4% $\pm$5.1). The liver tissue samples were obtained from non-munition-contaminated deer. (See Section 2.1.)

Figure 3 depicts chromatograms generated in the HPLC analysis of a liver samples from a BAAP deer. Figure 3.a is the HPLC chromatogram of the extract obtained from the liver tissue of deer No. 11 from BAAP to which no authentic compounds had been added. The two arrows indicate where 2,6-DNT and 2,4-DNT should appear on the chromatogram if they were present. Figure 3.b is

Figure 3. HPLC Chromatogram of Deer Liver Extract



the HPLC chromatogram of the same deer liver tissue spiked with 0.1 ppm of 1,3-DNB, 2,4-DNT and 2,6-DNT before extraction. The calculated recoveries of the spiked compounds are 94%, 74%, and 82%, respectively.

These data indicate that the extraction method performed well for the three compounds of interest in that (1) the recovery of spiked compounds was satisfactory and (2) the reproducibility of the system was very good for compounds present at the 0.1 ppm level in animal tissues.

3.3 SAMPLE ANALYSIS

A total of 103 separate analyses were performed on the various field-collected animal tissues to determine whether DNT was present. The number and type of samples processed are listed in Table 1. Each analysis consisted of the extraction of an individual tissue sample followed by HPLC and examination of the eluent for 2,4-DNT and 2,6-DNT. Each chromatogram was examined by Dr. Lee Shugart. No DNT was observed in any of the BAAP-deer samples at or above the 0.1 ppm level.

TABLE 1. NUMBER AND TYPE OF FIELD-COLLECTED SAMPLES PROCESSED FOR DINITROTOLUENE

Sample type	Samples[a]	Controls[b]	Spikes[c]	Total
Deer liver	24(12)	2(1)	6(6)	32
Deer muscle	24(12)	2(1)	1(1)	27
Deer heart	24(12)	2(1)	1(1)	27
Blank(no tissue)			17	17
Total	72	6	25	103

[a] Indicates number of tissue preparations analyzed. Number in parenthesis indicates number of animals from which these samples were derived.

[b] Samples from animals from non-contaminated sites.

[c] Samples to which known amounts of authentic compounds were added before processing.

3.4 STATISTICAL ANALYSIS OF DATA

In the results reported in this study, none of the tissues analyzed was found to contain 2,4-DNT or 2,6-DNT at or above 0.1 ppm. The following exercise was performed with the data obtained from BAAP site deer tissue (refer to Shugart et al. 1991 for details):

> For a deer population size of N = 430; a random sample size of n = 12; and with zero observed number of animals with an individual compound concentrations greater than the detection limit (DL) of the analytical procedure used; then from Wright's Tables, we find the upper 95% confidence limit on the proportion of the population that exceeds the DL to be 0.22. We are 95% confident that no more than 22% (93/430) of the deer population exceeds the DL for an individual compound). This confidence statement about the proportion of the population that exceeds the DL can be converted to a confidence statement about the proportion greater than the CL if we assume the distribution of DNT-related observations are lognormal with parameters (μ, σ^2) and that ln CL is $k^*\sigma$ units above ln DL. The upper limit on the proportion > DL may be converted to an upper limit on the proportion > CL, by finding the proportion of the distribution > $\mu+(0.78+k^*)\sigma$, where the value k^* is defined as $((\ln CL - \ln DL)/\sigma)$. For $\hat{\sigma} = 0.887$ (the estimate for σ is taken from Shugart, et al. 1991), and CL and DL in ppm, $k^* = (\ln 1.0 - \ln 0.1)/.887 = 2.302/.887 = \underline{2.60}$. Then from standard statistical tables on the proportion of the normal curve (one-tailed) that lies beyond a given normal deviate, we can say with 95% confidence that this proportion is 0.0004. Thus, no more than 0.04% of the 430 deer exceed the CL limit, or less than 1 deer.

4. DISCUSSION AND CONCLUSIONS

The main objective of the work detailed in this report was to determine whether deer on the Badger Army Ammunition Plant site, Badger, Wisconsin, contained the two isomers of DNT in various tissues (liver, muscle, and heart) at concentrations that would be unsuitable for human consumption.

On January 10, 1991, samples of liver, muscle, and heart from twelve deer taken at the BAAP site were received at ORNL. Over a period of several months, these samples were analyzed for DNT content.

The extraction procedure used for the isolation of the two isomers of DNT from the deer samples was a modification of one developed previously for other munition-like compounds (Shugart et al. 1991). It provided the following advantages:

1. Enrichment of the compounds of interest with a minimum of coextraction of extraneous matter that might interfere with the HPLC analysis.

2. Acceptable and reproducible recoveries of spiked authentic compounds.

The HPLC procedure was demonstrated to be satisfactory for the detection of the DNT compounds at the level of sensitivity required for this work (0.1 ppm in the tissue sample).

No DNT compounds were found at or above the detection limit in all of the tissues of the twelve deer taken from the BAAP site

or in deer from the control site. The statistical analysis of the data suggest that, with 95% confidence, no more than 93 animals from a total population estimated to be about 430 at the BAAP site have levels of have these compounds in excess of 0.1 ppm in their tissues and, further, that no more than 0.04% of the 430 animals would have these compounds at a concentration that exceeds 1.0 ppm. In essence, the significance of the statistical analysis is that less than one deer from the total population at the BAAP site would be contaminated with DNT at the 1.0 ppm level.

Regarding the statistical analysis of the data generated in this work it should be noted that

1. A CL (a toxicologically significant level that would preclude human consumption) has not been determined for DNT in deer tissue. However, based on previous studies with munitions-like compounds (trinitrotoluene and its metabolites) conducted for the U.S. Army (Shugart et al. 1991), a CL of about 1 ppm (1.0 mg/kg) of DNT in animal flesh appears reasonable.

2. In the absence of DNT compounds in sampled animals at the DL, the statistics were designed to quantify the confidence concerning absence of excessive contamination at the CL in the remaining population. In this regard, supplementary data analysis was used (Shugart et al. 1991) to support the assumption that the magnitude of σ (standard deviation of the distribution of a chemical in a population of exposed animals) can be described by a lognormal distribution.

ACKNOWLEDGEMENT

This work was performed under contract No. ERD-90-1001 at the Oak Ridge National Laboratory for the Olin Corporation, Baraboo, Wisconsin. The Oak Ridge National Laboratory is managed by the Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract DE-AC05-84OR21400. This is Environmental Sciences Division Publication No. 3802.

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Beauchamp, J. J., J. F. McCarthy, D. H. Rosenblatt, and L. R. Shugart. 1991. Statistical design for sampling and analysis of animal populations for chemical contamination. Risk Analysis, in press.

Shugart, L. R., W. H. Griest, E. Tan, C. Guzman, J. E. Caton, C.-H Ho, B. A. Tompkins. 1991. TNT metabolites in animal tissues. ORNL/M-1336. Oak Ridge National Laboratory, Oak Ridge, Tenn.

Wright, T. 1991. Exact confidence bounds when sampling from small finite universes-an easy reference based on the hypergeometric distribution. In: Lecture Notes in Statistics, Vol. 66. Springer-Verlag, New York.

APPENDIX A

DEER COUNT AT BADGER ARMY AMMUNITION PLANT

DEPARTMENT OF THE ARMY
BADGER ARMY AMMUNITION PLANT
BARABOO, WISCONSIN 53913

April 15, 1991

Commander's Representative

SUBJECT: Deer Count at Badger Army Ammunition Plant

Dr. Schugart
Oak Ridge National Laboratory
Post Office Box 2008
Oak Ridge, TN 37831

Dear Dr. Schugart:

The following is the deer count you requested to complete your report on the deer tissue sample sent in November of 1990.

The table contains the deer herd count post hunting season, the year the count was made, the harvest and the count of deer found dead on plant when available.

DEER HERD INFORMATION

Year	Herd Count Post Hunt	Hunting Harvest	Dead of Other Causes
1990	250 (estimated)	181	10
1989	282 (ground & helicopter)	205	2
1988	322 (ground & helicopter)	124	N/A
1987	153 (ground & helicopter)	117	N/A
1986	243 (ground & helicopter)	124	N/A

Please conract me at (608)356-5525 if there are any questions.

Sincerely,

David C. Fordham
Commander's Representative

APPENDIX B

STATISTICAL DESIGN FOR SAMPLING AND ANALYSIS OF ANIMAL POPULATIONS FOR CHEMICAL CONTAMINATION

Submitted for publication in:

Risk Analysis

Title:

Statistical Design for Sampling and Analysis of Animal Populations for Chemical Contamination

Authors:

John J. Beauchamp[1], John F. McCarthy[2], David H. Rosenblatt[3], and Lee R. Shugart[2]

[1]Engineering Physics and Mathematics Division, P.O. Box 2009, Oak Ridge National Laboratory, Oak Ridge, TN 37831-8083.

[2]Environmental Sciences Division, P.O. Box 2008, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6036.

[3]Health Effects Research Division, U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, MD, 21701-5010.

Abbreviated title:

Sampling And Analysis Design

Send correspondence to:

Dr. Lee R. Shugart

P.O. Box 2008

Oak Ridge National Laboratory

Oak Ridge, TN 37831-6036

ABSTRACT

A strategy for sampling of animal tissues and a statistical approach for analyzing data on body burdens of a parent chemical and its metabolites is presented such that the data may be evaluated in relation to the detection limit(s) of the analytical techniques used and the criterion levels established for acceptable tissue concentrations.

Key Words:

Body Burdens; Analysis Decision; Nondetectable Values

1. INTRODUCTION

Often it is desirable to analyze the tissue of animals, either wild or domesticated, living in an area polluted with known chemicals to establish whether or not they are free of excessive toxic contamination, especially if a potential exists for ingestion of meat containing these chemicals.

A statistical design is presented with the objective of deciding whether a chemical and its metabolites are likely to be present in the tissues of an animal population at or above a designated criterion level when only a portion of the population has been sampled. In the absence of such compounds in sampled animals at the detection level, the statistics are designed to quantify the confidence concerning absence of excessive contamination at the criterion level in the remaining population. A Sampling and Analysis Decision Tree is presented that takes these constraints into account.

Although animal tissue is highlighted as the chemically contaminated matrix for study in this paper, it should be emphasized that other environmental samples may be appropriate candidates for analysis by the statistical design introduced.

2. STRATEGY FOR SAMPLING AND ANALYSIS

2.1 Definitions

N	population size
n	number of animals analyzed (sample)

A	number of animals in population with levels of a chemical and/or its metabolites exceeding CL
a	number of animals in sample with levels of a chemical and/or its metabolites exceeding CL
a'	number of animals in sample with levels of a chemical and/or its metabolites exceeding DL
P^*	proportion of population exceeding CL of chemical (= A/N)
CL	critical level of chemical and/or its metabolites in animals
DL	detection limit for quantifying chemical and/or its metabolites
β	proportion of distribution covered by tolerance interval
γ	confidence level
μ and σ	pertain to the unknown mean and standard deviation, respectively, for the metabolite distribution
$\bar{y}$ and v	pertain to sample estimates of μ and σ, respectively
Y	random variable associated with the distribution of metabolites, usually a lognormally distributed variable
k'	constant multiplied times v, used in determining the upper limit of a β-content tolerance interval, i.e., the number of standard deviations above the mean
k^*	difference between ln CL and ln DL expressed in σ units
[C]	concentration of compound(s)

2.2 Sampling scenarios and statistical considerations.

The objective of the sampling of animals is to estimate P^*, the proportion of the total population of a species at a particular site that contains body burdens of a chemical and/or its metabolites above a "critical level" (CL). A statement about P^* derived from a statistical analysis of the data collected should have a stated degree of confidence based in part on the detection limit (DL) of the analytical techniques employed. Therefore, two sampling scenarios are described that allow for statistical analyses to be performed on the data in relation to the DL.

2.2.1. Scenario #1.

If the tissue levels of the chemical and metabolites are sufficiently high relative to the detection limit (DL) of the analytical technique employed, then the distribution of the metabolite concentrations can frequently be characterized by a normal or lognormal distribution. P^* can be estimated from the observed sample proportion >CL, i.e., by a/n. Using the sample estimates $\bar{y}$ and v, a tolerance interval can then be constructed for the proportion of the population >CL (i.e., P^*), and a statement can be made that we are $100\gamma\%$ confident that the proportion of the population >CL is no more than $1-\beta$ (Guttman 1970). In this scenario, it is assumed that the population size (N) is large relative to the sample size (n). If the normality assumption is appropriate for the observed or transformed (e.g., log) metabolite concentrations, then $\bar{y}$ and v are the sample mean and standard deviation, respectively, calculated from the observed or transformed observations. The one-sided β-content tolerance interval is of the form $(-\infty, \bar{y}+k'v)$ and, for the appropriate value of k', a statement of the following form can be made:

We are $100\gamma\%$ confident that at least $100\beta\%$ of the distribution of y is less than $\bar{y} + k'v$.

Tables giving the values of k′ for different combinations of γ and β are given in Guttman (1970, Table 4.6) or Odeh and Owen (1980, Table 1.4).

When some of the observations in the sample are censored (i.e., the observed value is known only to be less than a known detection limit, but its actual value is unknown), it is necessary to use alternatives to the usual sample mean and variance to obtain estimates of the population parameters needed in the previously-described tolerance interval. Many alternative methods are available to estimate the parameters—ranging from graphical methods (Travis and Land 1990) and ad hoc computational methods (Haas and Scheff 1990, Helsel and Cohn 1988, Gilliom and Helsel 1986, Newman et al. 1989, Helsel 1990, Newman and Dixon 1990) all the way to computationally-intensive maximum likelihood methods developed by Cohen (1963, 1976) for progressively censored samples. Although the graphical and ad hoc procedures are easy to implement, they do not make the most efficient use of the data when it is reasonable to assume a particular form for the underlying distribution of the data, e.g., normal or lognormal. Computer programs (e.g., the LIFEREG procedure in SAS [1989]) are readily available to obtain the maximum likelihood estimates. Therefore, the maximum likelihood method or some modification of it is recommended for obtaining estimates of the mean and variance when censored data are available. Once these estimates are obtained, they can be substituted in the place of $\bar{y}$ and v in the above tolerance limit construction and in the subsequent calculations.

Before determining the sample size required to estimate P^* with a specified confidence, it is necessary to determine the relation between $\bar{y}$, v, k′, and CL. If CL is a critical or action level for $\bar{y}$, then once we have a random sample, we may solve

$$\bar{y} + k' v = CL$$

for

$$k' = \frac{CL - \bar{y}}{v} .$$

From this value of k', for a fixed value of γ we can determine the proportion of the population that is greater than CL. For example, if CL = 4, $\bar{y}$ = 2, and v = 0.75 from a sample of size n = 10, then

$$k' = \frac{4-2}{0.75} = 2.67$$

If γ = 0.95 and n = 10, then from Guttman (1970, Table 4.6) or Odeh and Owen (1980, Table 1.4) we have

(1) for β = 0.90, k' = 2.355

(2) for β = 0.95, k' = 2.911

Since the observed value of k'(=2.67) falls between these two values, a lower bound on β is given by 0.90, i.e., β >0.90 or 1 - β <0.10. In a similar manner, if γ = 0.90, then a lower bound on β is 0.95, i.e., β >0.95 or 1 - β <0.05. Therefore, in the first case, we would say we are 95% confident that no more than 10% [= 100(1 - β)] of the distribution is greater than CL, i.e., 95% confident that 100 P^* <10%. In the second case, we are 90% confident that no more than 5% [=100 (1 - β)] of the distribution is greater than CL, i.e., 90% confident that 100 P^* <5%.

In order to estimate the sample size required to draw conclusions about P^*, using β, with acceptable statistical confidence, it is necessary to determine a range on $\bar{y}$ and v from which a range on k' can be obtained. From the minimum value of this range on k', we can find, for a

fixed value of γ, the sample size needed to have β greater than a specified value. For example, if we assume, or have reason to believe, that the ranges of $\bar{y}$ and v will yield a minimum anticipated value of k' to be 3.0, and if $\gamma = 0.95$, then for

(i) $\beta > 0.95$, from Guttman (1970, Table 4.6) or Odeh and Owen (1980, Table 1.4) we have for $n \leq 9$ that $k' > 3.0$, and for $n \geq 10$ that $k' < 3.0$. Therefore, a sample size of $n \approx 10$ would be needed for this combination of k', γ, and β.

(ii) $\beta > 0.99$, similar steps result in $n \approx 35$.

Similar calculations are repeated for other combinations of k', γ, and β to produce the results in Table 1. The following example shows how Table 1 may be used to estimate the sample size needed to estimate P^*.

Example: Assume that we anticipated the CL to be not more than 3 (= k') standard deviation units above $\bar{y}$, i.e., CL - $\bar{y}$ is $\leq k'v$. From Table 1, a minimal sample of 6-7 animals would be required if we want to be 90% confident ($\gamma = 0.90$) that no more than 5% ($1-\beta = 0.05$) of the distribution of metabolite concentrations (in log-units) would be above $\bar{y} + 3$ v, i.e., if we want to be 90% confident that $P^* < 0.05$.

When a = 0, it is possible that the upper tolerance limit ($\bar{y} + k'v$) would be >CL. When a >0, it is unlikely that $\bar{y} + k'v$ would be <CL.

2.2.2. Scenario #2.

When the concentrations of chemical and/or metabolites of concern in the tissues of the animals are below the DL of the analytical technique employed in more than 50% of the sample observations, it is difficult to obtain precise estimates of μ and σ. In that case, the objective of the sampling is to demonstrate that, with some statistical certainty, no more than x% of the population exceeds CL (e.g., P^* should probably be no more than 5%). Wright (1991) presents the development of a method, along with the necessary tables, to obtain confidence limits on P^* when we assume we are sampling from a hypergeometric distribution. Therefore, we can approach this problem based on the tables of Wright (1991) for sampling from finite populations. Furthermore, we will assume that the body burdens within the population are distributed lognormally.

For example, let us assume a population size of N = 400 and a random sample of size n = 10; then if the observed number of animals with metabolite concentrations greater than the DL is zero, we may use Wright's tables (with $a'=0$) to find the upper 95% confidence limit on the number in the population that exceeds DL to be 102, which translates into a corresponding limit on the proportion of the population that exceeds the DL to be 0.26 (102/400) (i.e., we are 95% confident that no more than 26% of the population exceeds DL). Furthermore, by assuming knowledge about the difference between DL and CL we can convert the confidence statements about the proportion greater than DL to statements about the proportion of the population greater than CL. For a lognormal distribution, $Y = \ln X$ is assumed to have a normal distribution with mean μ and variance σ^2 and the point that cuts off 0.26 (the above upper confidence limit)

of the upper portion of this distribution may be obtained from any table of area for the normal distribution and is given by ($\mu + 0.64\sigma$). If the additional assumption is made that ln CL is $k^*\sigma$ units above ln DL, then the upper limit on the proportion >DL may be converted to an upper limit on the proportion >CL, by finding the proportion of the distribution $>\mu+(0.64+k^*)\sigma$. The value of k^* is defined as ((ln CL - ln DL)/σ). For example, if $k^* = 1$, the proportion of the normal distribution $>\mu + (0.64 + 1)\sigma$ is equal to 0.05. Other combinations of n, a', and k^* are used to repeat the above procedure and produce the values in Table 2. Other values of N could also be considered by using a different entry in the tables by Wright (1991).

This formulation demonstrates that the statistical certainty of any statements about estimates of P^* will depend on the following variables (Table 2):

1. The number of animals sampled (n) and the number exceeding the DL or CL;

2. The magnitude of the difference between CL and DL; and

3. Assumptions about the magnitude of σ.

Example: As an illustration of the use of Table 2, assume that the variance is one log unit ($\sigma = 1$) and the CL is one log unit greater than DL, then $k^* = 1$. If the DL is lowered an additional log unit, then $k^* = 2$. Assume that we sample 10 (= n) animals from a population of 400 (= N) animals and find none (a' = 0) with chemical levels above the DL. If $k^* = 1$ (i.e., CL is one log unit >DL), then, using the results in Table 2, we can state with 95% certainty that P^* is not more than 5%. If we decrease the DL by one log unit, k^* becomes 2.0, and there still

were no ($a' = 0$) animals above the DL, we are assured that no more than 0.4% of the population is above CL. If, however, the lower DL enables us to detect measurable levels of metabolites in one animal (Table 2, $n=10$, $a'=1$, $k^*=2.0$), then we are 95% confident that P^* is no more than 1% of the population. In this formulation, the ability to make statements about P^* depends on the number of animals sampled and the difference between CL and DL.

This analysis is also sensitive to assumptions about the magnitude of σ, which cannot be experimentally determined if only a small proportion of the population exceeds DL. Therefore, prior knowledge about the magnitude is required. This information may be obtained from data of animals or chemicals anticipated to respond in a similar manner.

In the design of this sampling strategy for either scenario, two key variables can be controlled:

1. Number of animals collected. For large animals such as deer, there is, realistically, an upper limit on the "reasonable" number of animals that can be sampled. The number of animals that need to be sampled to provide acceptable tolerance limits on P^* cannot be determined a priori, but preliminary estimates suggest that approximately 10-20 animals may be sufficient (Table 2), and this is a number that seems reasonable from a logistical perspective. However, much larger numbers of small animals can be sampled.

2. The DL of the analysis for the chemical can be improved by increasing the volume of tissue extracted. This approach is applicable to animals of large size. However, the extent

to which the DL could be improved for small species is questionable, and depends mainly upon the amount of tissue required for the analytical techniques used.

3.0 SAMPLING AND ANALYSIS DECISION TREE

Based on the considerations discussed above, we propose the following strategy, which is summarized as a "Decision Tree" in Figure 1:

Collect appropriate samples from designated animals. Extract and analyze chemical and/or its metabolites using appropriate methodologies. Evaluate the results:

1. If more than 50% of the samples >DL (sampling scenario #1), then estimate μ and σ^2 for each population and tissue. Estimate proportion of the population greater than CL and estimate tolerance limit on population:

   If the portion of the population >CL is <u>acceptable</u> (e.g., $P^* < 5\%$ of the population), and the tolerance limits are acceptable, then conclude that no health danger exists and end the study.

   If the portion of the population >CL is <u>unacceptable</u> and the tolerance limit are acceptable (e.g high confidence that $P^* > 5\%$ of the population) accept that the animals present a potential health danger and end the study.

If the confidence in an estimate of the P^* is low (e.g., $\gamma < 0.9$), then the statistical confidence of the estimate can be improved only by increasing the sample size. If additional animal samples are available, these can be analyzed to increase the confidence of the estimate of P^*. It must be recognized that a cost/benefit decision must be made prior to sampling: a balance must be achieved between the effort involved in collecting (and potentially not needing) additional animals, compared to the benefits of being assured that the final analyses will permit statistically acceptable statements about P^*.

2. If more than 50% of the samples <DL (sampling scenario #2), but the upper limit on P^* is <u>acceptable</u> (i.e., there is greater than 95% confidence that $P^* \leq 5\%$ of the population, given a scientifically defensible assumption on the value of σ and based on an analysis as illustrated in Table 2), then conclude that no health danger exists and end the study.

3. If more than 50% of the samples <DL (sampling scenario #2), but the upper limit on P^* is <u>unacceptable</u> (i.e., it cannot be stated with 95% confidence that $P^* \leq 5\%$ of the population), then two choices exist: accept that the animals may present a potential health danger and end the study, or improve confidence in the conclusion by gathering more data to improve statistical power.

If more data need to be analyzed, different strategies are employed for different species (Table 2):

For small animals, the mass of tissue available for analysis is limited, but more animals are available for analysis. Therefore, the statistical power will be increased by analyzing some or all of the animals held in reserve.

For large animals, sample volume is not limited, so we will decrease DL by extracting the larger volume tissue samples held in reserve. Animals held in reserve can also be analyzed to increase sample number, but inspection of Table 2 suggests that a decrease in DL (which increases k^*) will have a greater effect in improving statistical confidence.

4.0 SUMMARY

Pollutants in the environment may pose risks to human health via contaminated food sources. Since many pollutants may be presented in biota at concentrations below the detection limits of existing methods, analytical analyses could provide nondetectable values. A strategy for sampling and statistical analysis is present that accommodates the detection limits of the analytical technique used and the criterion levels established for tissue concentrations.

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Table 1. Sample Sizes for Combinations of γ, β, and k'

(a) $\gamma = 0.90$

k'	$\beta = 0.90$ $1-\beta = 0.10$	$\beta = 0.95$ $1-\beta = 0.05$	$\beta = 0.99$ $1-\beta = 0.01$
1.5	76-77	>1000	>1000
2	11-12	42	>1000
2.5	5-6	11-12	230-240
3	4-5	6-7	22-23
3.5	3-4	4-5	10-11
4	3-4	3-4	6-7

(b) $\gamma = 0.95$

k'	$\beta = 0.90$ $1-\beta = 0.10$	$\beta = 0.95$ $1-\beta = 0.05$	$\beta = 0.99$ $1-\beta = 0.01$
1.5	120-130	>1000	>1000
2	17-18	66-67	>1000
2.5	8-9	16-17	375
3	6	9-10	34-35
3.5	4-5	6-7	15-16
4	4	5-6	9-10

(c) $\gamma = 0.99$

k'	$\beta = 0.90$ $1-\beta = 0.10$	$\beta = 0.95$ $1-\beta = 0.05$	$\beta = 0.99$ $1-\beta = 0.01$
1.5	240-250	>1000	>1000
2	31-32	120-130	>1000
2.5	15-16	30-31	700-800
3	10-11	16-17	65-66
3.5	7-8	11-12	28-29
4	6-7	8-9	17-18

Table 2. Upper 95% Confidence Limit On P^*

for a Population at Size N = 400[1]

n	a'	k^*		
		0.5	1.0	2.0
10	0	0.13	0.05	0.004
	1	0.22	0.10	0.01
	5	0.61	0.41	0.11
20	0	0.06	0.02	0.001
	2	0.14	0.06	0.005
	10	0.50	0.31	0.07
30	0	0.03	0.01	0.0004
	3	0.11	0.04	0.003
	15	0.46	0.28	0.06

[1] Derived from information in Wright (1991).

LEGEND

Figure 1. Graphic representation of a sampling and analysis decision tree.

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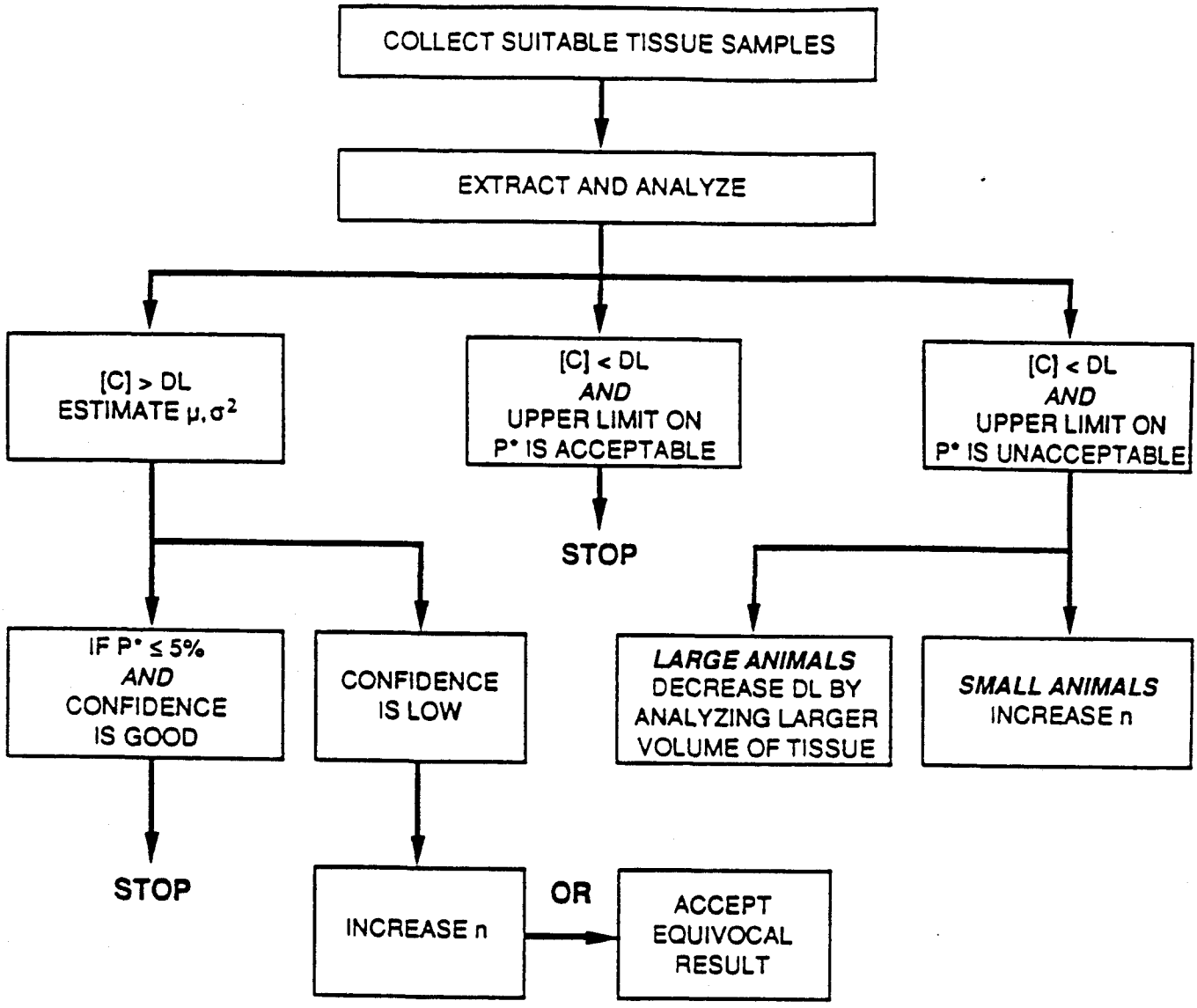

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