EPA 903/9-80-004

U.S. ENVIRONMENTAL PROTECTION AGENCY

METHOD OF STANDARD ADDITIONS

AND EFFECTS OF DILUTION

May 1980

Annapolis Field Office
Region III
U.S. Environmental Protection Agency

Method of Standard Additions

and Effects of Dilution

May 1980

Joseph Lee Slayton
E. Ramona Trovato

Annapolis Field Office

Region III

U.S. Environmental Protection Agency

The method of standard additions is useful when the matrices

of standards and samples differ significantly. The method of additions

may involve additions of small quantities of the analyte to the sample

with no significant dilution of the sample or cases which involve

significant sample dilution. In general, a plot is made of absorbance

versus added concentration and the value of the X-intercept is taken

as the analyte concentration.

The method of standard addition eliminates interferences that

cause constant multiplicative errors in the concentration of analyte

measured. As an example of this type of interference, consider a

sample with a true analyte concentration of 1.0 ppm. The analyst

assays the sample and determines a concentration of 0.5 mg/1 indicating

an interference factor of .5. (This is a negative interference since

the interference factor is less than 1.) The sample is spiked with

small quantities of the analyte to produce an added concentration of

1.0 ppm and 2.0 ppm and values of 1 ppm and 1.5 ppm, respectively,

are obtained. From these results the analyst decides there is an

interference and plots the data (Figure 1).

Figure 1.   Data and Plot of Method of Standard Additions

Concentration of analyte added after dilution by sample (mg/1)	*Measured Concentration (mg/1)	**Measured Absorbance
0	.5	.05
1	1.0	.10
2	1.5	.15

*This represents an interference factor of .5.
**The standard curve had a slope of .1 and a y-intercept of zero.
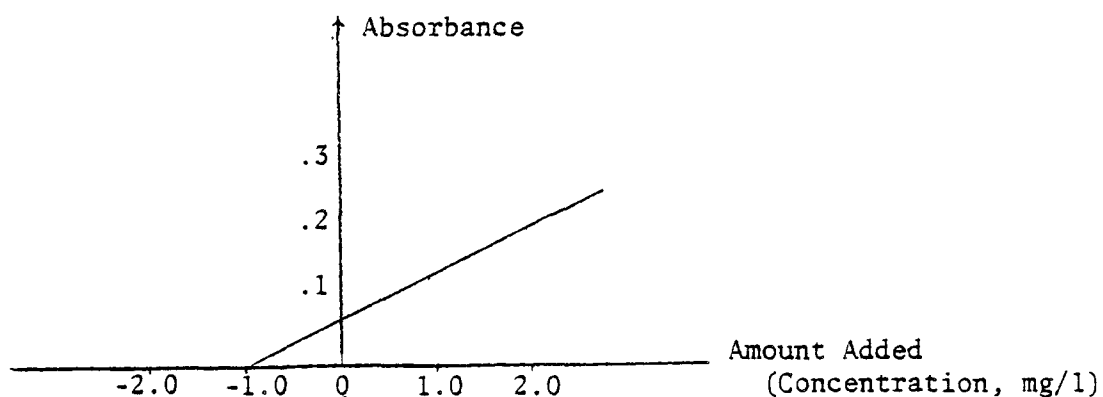
Scale of negative concentration
values and positive concentration
values must be the same.

The extrapolation of the line to the X-intercept generates the actual

concentration of the sample (ignoring the negative sign).

The addition of small known quantities of the analyte or "spiking"

to determine sample concentration is based upon the Beer's Law

relationship: the absorbance is directly proportional to the concentration

of the analyte present   (Figure 2) .

Figure 2. Plot of Absorbance vs Concentration with Application of Beer's Law

Absorbance

Ab_1

Ab_0

Concentration
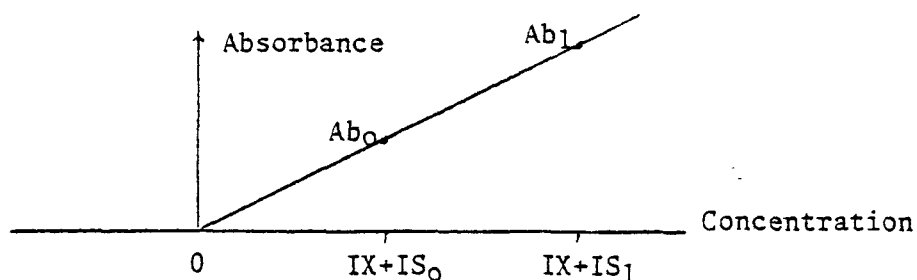
0      $IX + IS_0$ $IX + IS_1$

Beer's Law:

$$\frac{Ab_0}{IX + IS_0} = \frac{Ab_1}{IX + IS_1}$$

or

$$X = \frac{(Ab_0)(S_1)}{Ab_1 - Ab_0} \cdot \frac{I}{I}$$

or

$$X = Ab_0 S_1 / (Ab_1 - Ab_0)$$

where: Ab_0 = Absorbance of solution with concentration = $IX + IS_0$

Ab_1 = Absorbance of solution with concentration = $IX + IS_1$

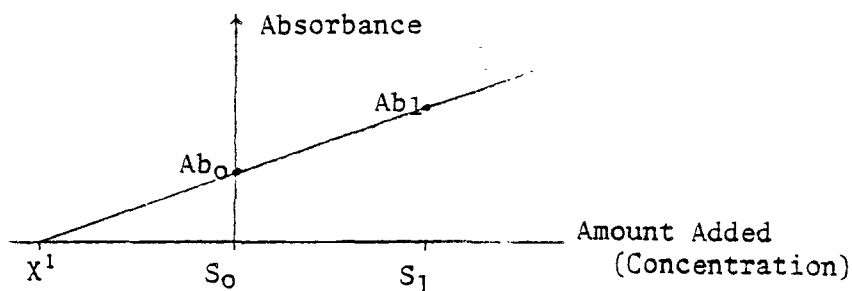I = Interference factor (constant multiplicative error)

S_0 = 0 spike addition = 0

S_1 = Concentration of analyte added after dilution by the total volume (sample volume + spike volume)

X = Concentration of analyte in sample

The sample concentration may be determined by solving for X in the proportion (Figure 2). The method of standard additions plot may be generalized (Figure 3):

Figure 3. Generalized Plot of Method of Standard Additions

Absorbance

Ab_1

Ab_0

X^1 S_0 S_1

Amount Added (Concentration)

$y = mX + b$

at $y = 0$

$X^1 = -b/m$

$b = Ab_0$

$m = (Ab_1 - Ab_0) / S_1$

$X^1 = -Ab_0 S_1 / (Ab_1 - Ab_0)$

where: Ab_0 = Absorbance of solution with zero analyte addition

Ab_1 = Absorbance of solution associated with addition of spike of S_1

S_0 = 0 spike addition

S_1 = Concentration of analyte added after dilution by the total volume (sample volume + spike volume)

X^1 = Method of standard addition determined concentration of analyte in sample

3.

Again, a linear relationship based upon Beer's Law is assumed. A linear
relation is defined: $y = mX+b$; where y is the absorbance, X is the concentration,
m is the slope, and b is the y-intercept. At the X-intercept, $y = o$ and $X^1 =$
$-b/m$, or $X^1 = -Ab_oS_1/Ab_1-Ab_o$. The extrapolation generates the same numerical
value (but negative in sign) as the true result and is therefore applicable to
additions of small volumes of analyte. The interference free (true) result
was obtained from the Beer's Law relation because of the constant nature of
the interference.

A problem arises in using the extrapolation method when significant
and varying dilutions of the sample by the addition of the spike are involved.
The spiked samples are prepared by adding a significant volume of analyte and
diluting to a pre-set volume with the sample (i.e., 1. establish a pre-set
volume, for example, 100 ml; 2. for the first spiked sample, add 2 ml of
analyte of a known concentration and add 98 ml of sample to bring the total
volume to 100 ml; 3. for the second spiked sample, add 4 ml of analyte of the
same known concentration and add 96 ml of sample to bring the total volume
to 100 ml; etc.).The results obtained from this method of standard additions
are incorrect because the concentration of the interferent varies in each
spiked sample aliquot. In order to accurately determine the analyte
concentration, the concentration of the interferent must be kept constant.
This can be accomplished by following the EPA method of standard additions.

The results of the EPA method of standard additions outlined in the 1974 and 1979 editions of the EPA Manual of Methods of Chemical Analysis of Water and Wastes* may be effected by dilution. The EPA method requires that a constant volume of sample be added to a constant volume of standards and blank. If the volume of sample consistently used is V and the volume of standards and blank is consistantly v where $V \neq v$, this experiment may be generalized to that presented in Figures 4(1) and 4(2).

*Metal's section page 12, 1979 EPA Manual of Methods of Chemical Analysis of Water and Wastes

Figure 4(1). <u>Correct result</u> obtained using Beer's Law coupled with the Dilution and Interference Factors and Using the Procedure outlined by EPA

V	V	V	V
v blk	v std #1	v std #2	v std #3

Absorbance

Ab_1

Ab_0

0

Concentration
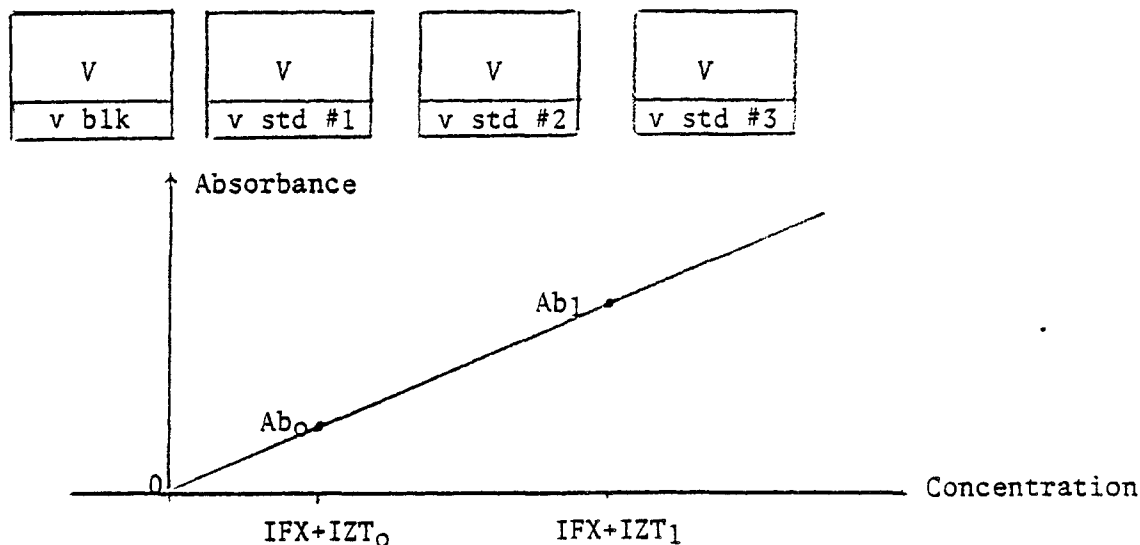
$IFX + IZT_0$ $IFX + IZT_1$

$$\frac{Ab_0}{IFX + IZT_0} = \frac{Ab_1}{IFX + IZT_1}$$

or $X = \dfrac{Z}{F} \cdot \dfrac{-Ab_0T_1}{Ab_0 - Ab_1}$

but; $Z/F = v/V$

therefore; $X_{True} = \dfrac{v}{V} \cdot \dfrac{Ab_0T_1}{Ab_1 - Ab_0}$

where: V = Volume of sample
v = Volume of standard or blank
$F = V/(V+v)$
$Z = v/(V+v)$
I = Any interference factor
T_0 = Concentration of the analyte in the blank = 0
T_1 = Concentration of the analyte in standard #1 ($S_1 = ZT_1$)
Ab_0 = Absorbance associated with the concentration: $IFX + IZT_0$
Ab_1 = Absorbance associated with the concentration: $IFX + IZT_1$
X = Sample concentration

Figure 4(2). Incorrect result obtained using the Method of Standard
Additions as outlined by EPA, where $V \neq v$, (an example
is included in the appendix).
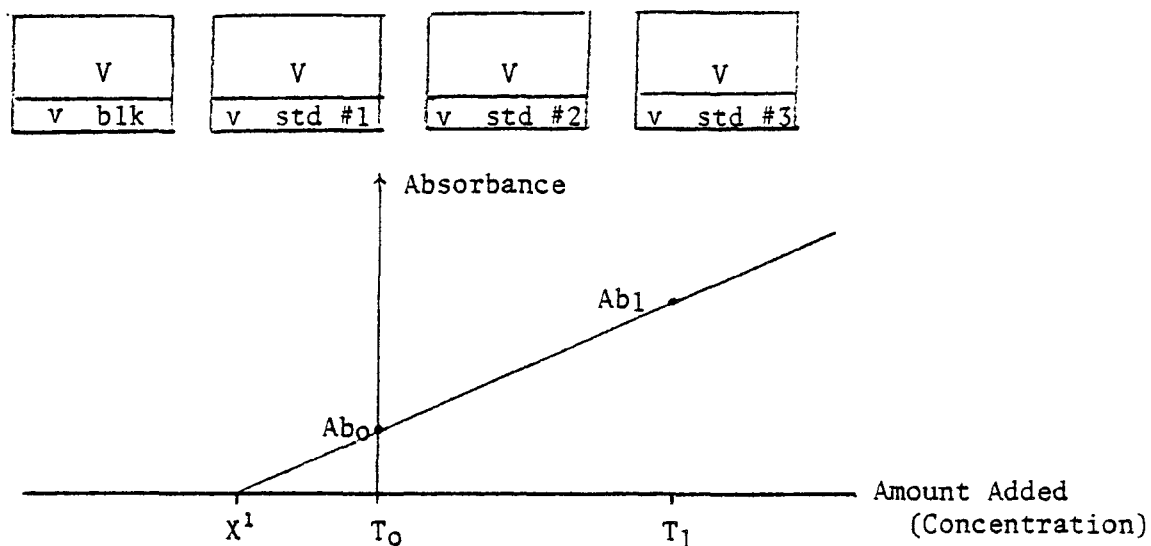
$$y = mX^1 + b$$
$$m = \Delta y/\Delta X = (Ab_1 - Ab_0)/T_1 - T_0$$
$$= (Ab_1 - Ab_0)/T_1$$
$$b = \text{y-intercept} = Ab_0$$
at y=o, $X^1 = -b/m$
therefore; $X^1 = (-Ab_0) \dfrac{T_1}{Ab_1 - Ab_0}$

$$X^1 = -\dfrac{Ab_0 T_1}{Ab_1 - Ab_0}$$

where: V = Volume of sample
v = Volume of standard or blank
T_0 = Concentration of the analyte
in the blank = 0
T_1 = Concentration of the analyte
in standard #1

Ab_0 = Absorbance associated with
zero analyte addition
Ab_1 = Absorbance associated with
addition of a concentration
of T_1
X^1 = Method of standard addition
determined concentration of
analyte in sample

These results indicate that if $v \neq V$, this method of standard additions does not yield accurate results. The results of extrapolation may be corrected by a factor, $-(v/V)$:
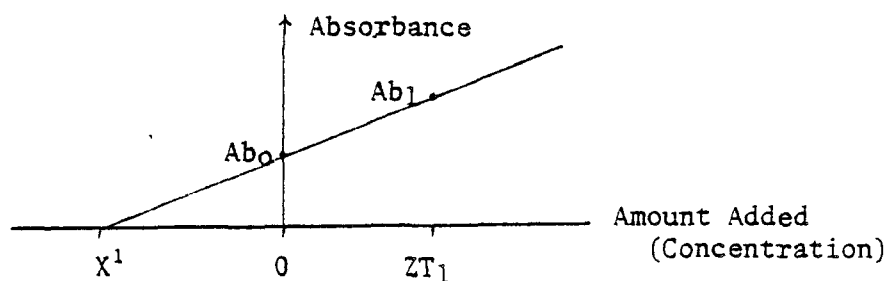
$$X_{True} = (X^1)(v/V)(-1)$$

If the "Amount Added" is interpreted as the concentration of standard corrected for dilution by the sample, the true result remains:

$$X_{True} = \frac{Z}{F} \frac{Ab_0 T_1}{Ab_1 - Ab_0}$$

the extrapolation becomes:

$$X^1 = \frac{-Ab_0 \ ZT_1}{Ab_1 - Ab_0} \qquad \text{(See Figure 5)}$$

Figure 5. Incorrect result obtained using the Method of Standard
Additions when Dilution of the Sample by the Spike is
Disregarded ($V \neq v$)

$y = mX + b$
$m = \Delta y/\Delta X = (Ab_1 - Ab_0)/ZT_1$
$b = Ab_0$
at $y = 0$, $X^1 = -b/m$
$X^1 = \dfrac{(-Ab_0 \ ZT_1)}{Ab_1 - Ab_0}$

where:
V = Volume of sample
v = Volume of standard or blk
F = $V/(V+v)$
Z = $v/(V+v)$
T_1 = Concentration of the analyte in standard #1 ($S_1 = ZT_1$)
Ab_0 = Absorbance associated with zero standard addition
Ab_1 = Absorbance associated with addition of a concentration of ZT_1
X^1 = Method of standard addition determined concentration of analyte in sample

The result of the extrapolation, $X^1 = \dfrac{(-Ab_0)(ZT_1)}{Ab_1-Ab_0}$, may be corrected

by a factor: $X_{True} = (X^1)\left(\dfrac{-1}{F}\right)$, where $F = V/(v+V)$.

The EPA method of standard additions yields accurate results

when $v = V$. In this case $F = \dfrac{V}{v+V} = Z = \dfrac{v}{v+V}$ and Beer's Law with

correction for dilution yields the result:

$$\dfrac{Ab_0}{IFX} = \dfrac{Ab_1}{IFX +IFT_1} \qquad\text{or}\qquad X_{True} = \dfrac{Ab_0T_1}{Ab_1-Ab_0}$$

which is the same result obtained from a plot of absorbance vs the

concentration of the analyte in the added standard (See Figure 6).

<u>Figure 6</u>.   Plot of Absorbance vs Concentration Added when V=v
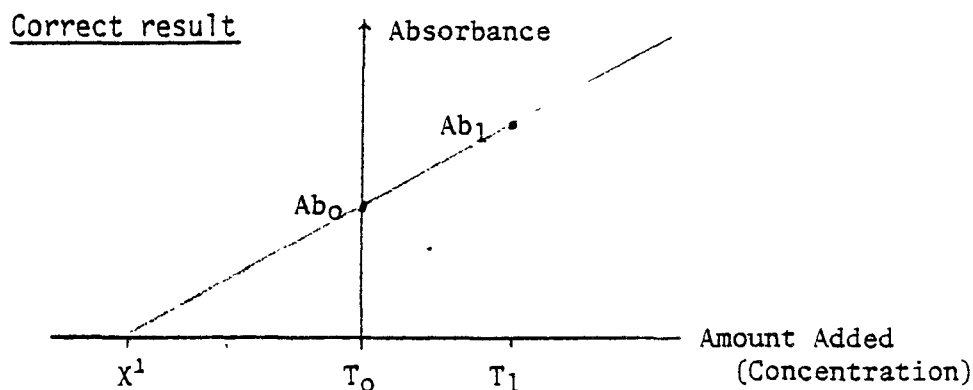
$y = mX+b$
at $y = 0$, $X^1 = -b/m$
  $b = Ab_0$
  $m = \Delta y/\Delta X = (Ab_1 - Ab_0)/T_1$
  $X^1 = (-Ab_0)(T_1) / (Ab_1 - Ab_0)$
  $X_{True} = (X^1)(v/V)(-1)$ and $v = V$;
  $X_{True} = (X^1)(-1)$

where: V = Volume of sample
        v = Volume of standard or blank
        T_1 = Concentration of the analyte in standard #1
        Ab_0 = Absorbance associated with zero standard addition
        Ab_1 = Absorbance associated with addition of a concentration of T_1
        X = True concentration of analyte in sample
        X^1 = Method of standard addition determined concentration of analyte in sample

9.

The EPA method of standard additions does not account for the dilution of sample by the standard except when:

1. The volume of sample used is the same as the volume of standard, $V = v$.

2. Zero analyte was initially present, $Ab_0 = 0$.

3. No dilution of sample by standard occurs (insignificant volumes, e.g. microliter of standard and blank are used and diluted to a constant volume with sample).

If the volumes of sample and standard are significant and unequal, the extrapolation will only yield accurate results if a correction factor is employed (an example is included in the Appendix).

In conclusion, the method of standard addition does not necessarily correct for dilutions resulting from the addition of the analyte.

A 1.0 mg/1 phenol reference sample was analyzed using the 4-amino antipyrine method. The results obtained from a standard calibration curve and from the method of standard additions follow.

Standard Calibration Curve

Concentration (mg/1)	Absorbance	
0	.000	
.2	.029	
.5	.070	
.7	.098	correlation coefficient = .999
1.0	.142	slope = .140 y-intercept = -.000346
2.0	.272	
3.0	.421	
4.0	.562	

The reference sample had an absorbance of 0.142 which corresponds to 1.0 mg/1 based upon the standard calibration curve.

Standard Method of Additions

Twenty milliliters of sample were placed into each of four flasks. To flask #1, 80 ml of deionized water were added. To flask #2, 80 ml of a 0.5 mg/1 phenol solution were added. To flask #3, 80 ml of a 1.0 mg/1 phenol solution were added. To flask #4, 80 ml of a 2.0 mg/1 phenol solution were added. The concentrations added and absorbances obtained are tabulated in Table I.

Table I

Flask No.	Concentration Added (mg/1)	Absorbance	
1	0	.027	
			correlation coefficient = .9999
2	.5	.084	slope = .112
			y-intercept = .027
3	1.0	.138	at y = o, x = -.240
4	2.0	.252	

The result obtained from a plot of the data in Table I is 0.240 mg

phenol/1. This value is incorrect due to the dilution of the sample by the

spike. The actual concentration may be obtained by multiplying the value

of the x-intercept by v/V (see Equation 1), where v is the spike volume and

V is the sample volume.

Actual Concentration = (Value of the x-intercept)(-1)(v/V)   Equation 1

In this example, the actual concentration = (-.240)(-1)(80/20) = 0.96 mg/1.

Alternatively, the concentrations of the standards added may be corrected

for dilution by the sample. In Table II, the added concentrations corrected

for dilution by the sample and corresponding absorbances are listed. (Tables I

and II are identical except that the concentrations added have been corrected

for dilution by the sample in Table II).

Table II

Flask No.	Added Conc. Corrected for Dilution (mg/1)	Absorbance	
1	0	.027	
			correlation coefficient = .999
2	.4	.084	slope = .140
			y-intercept = .027
3	.8	.138	at y = o,x = -.192
4	1.6	.252	

The result obtained from a plot of the data in Table II is 0.192 mg/l. Again an incorrect result is generated. The actual value may be obtained by multiplying the x-intercept by $(v+V)/v$, where v is the spike volume and V is the sample volume (see Equation 2).

Actual Concentration = (Value of the x-intercept)$(-1)(v+V)/v$ Equation 2

In this example, the actual concentration = $(-.192)(-1)(100/20)$ = 0.96 mg/l.

13.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA 903/9-80-004	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE METHOD OF STANDARD ADDITIONS AND EFFECTS OF DILUTION		5. REPORT DATE May 1980
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J. L. Slayton and E. R. Trovato		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Annapolis Field Office, Region III U.S. Environmental Protection Agency Annapolis Science Center Annapolis, Maryland 21401		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS same		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA/903/00

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The basis and limitation of the Method of Standard Addition is discussed
with particular emphasis placed on the effects of dilution often encountered
in using this analytical tool. Dilutions employed when using Standard Additions
may effect the sample matrix. This problem may be avoided by maintaining a
constant sample dilution. If significant dilution of the sample is involved,
a dilution factor must be applied to obtain the correct result by the Method
of Standard Additions.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Standard Addition Beer's Law	Interference Spike	

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 13
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

INSTRUCTIONS

1. **REPORT NUMBER**
   Insert the EPA report number as it appears on the cover of the publication.

2. **LEAVE BLANK**

3. **RECIPIENTS ACCESSION NUMBER**
   Reserved for use by each report recipient.

4. **TITLE AND SUBTITLE**
   Title should indicate clearly and briefly the subject coverage of the report, and be displayed prominently. Set subtitle, if used, in smaller type or otherwise subordinate it to main title. When a report is prepared in more than one volume, repeat the primary title, add volume number and include subtitle for the specific title.

5. **REPORT DATE**
   Each report shall carry a date indicating at least month and year. Indicate the basis on which it was selected (e.g., date of issue, date of approval, date of preparation, etc.).

6. **PERFORMING ORGANIZATION CODE**
   Leave blank.

7. **AUTHOR(S)**
   Give name(s) in conventional order (John R. Doe, J. Robert Doe, etc.). List author's affiliation if it differs from the performing organization.

8. **PERFORMING ORGANIZATION REPORT NUMBER**
   Insert if performing organization wishes to assign this number.

9. **PERFORMING ORGANIZATION NAME AND ADDRESS**
   Give name, street, city, state, and ZIP code. List no more than two levels of an organizational hierarchy.

10. **PROGRAM ELEMENT NUMBER**
    Use the program element number under which the report was prepared. Subordinate numbers may be included in parentheses.

11. **CONTRACT/GRANT NUMBER**
    Insert contract or grant number under which report was prepared.

12. **SPONSORING AGENCY NAME AND ADDRESS**
    Include ZIP code.

13. **TYPE OF REPORT AND PERIOD COVERED**
    Indicate interim final, etc., and if applicable, dates covered.

14. **SPONSORING AGENCY CODE**
    Leave blank.

15. **SUPPLEMENTARY NOTES**
    Enter information not included elsewhere but useful, such as: Prepared in cooperation with, Translation of, Presented at conference of, To be published in, Supersedes, Supplements, etc.

16. **ABSTRACT**
    Include a brief (200 words or less) factual summary of the most significant information contained in the report. If the report contains a significant bibliography or literature survey, mention it here.

17. **KEY WORDS AND DOCUMENT ANALYSIS**
    (a) DESCRIPTORS - Select from the Thesaurus of Engineering and Scientific Terms the proper authorized terms that identify the major concept of the research and are sufficiently specific and precise to be used as index entries for cataloging.

    (b) IDENTIFIERS AND OPEN-ENDED TERMS - Use identifiers for project names, code names, equipment designators, etc. Use open-ended terms written in descriptor form for those subjects for which no descriptor exists.

    (c) COSATI FIELD GROUP - Field and group assignments are to be taken from the 1965 COSATI Subject Category List. Since the majority of documents are multidisciplinary in nature, the Primary Field/Group assignment(s) will be specific discipline, area of human endeavor, or type of physical object. The application(s) will be cross-referenced with secondary Field/Group assignments that will follow the primary posting(s).

18. **DISTRIBUTION STATEMENT**
    Denote releasability to the public or limitation for reasons other than security for example "Release Unlimited." Cite any availability to the public, with address and price.

19. & 20. **SECURITY CLASSIFICATION**
    DO NOT submit classified reports to the National Technical Information service.

21. **NUMBER OF PAGES**
    Insert the total number of pages, including this one and unnumbered pages, but exclude distribution list, if any.

22. **PRICE**
    Insert the price set by the National Technical Information Service or the Government Printing Office, if known.