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CIBA-GEIGY

Textile Products Division

CIBA-GEIGY Corporation
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Contains NO CB

8EHQ-92-12282
88920010494
INIT

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October 9, 1992

**CERTIFIED MAIL
RETURN RECEIPT REQUESTED**

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M. Street, SW
Washington, DC 20460

Attention: Section 8(e) Coordinator (CAP Agreement)

RE: 8E CAP - 0024

Dear Section 8(e) Coordinator:

Enclosed are triplicate copies of two studies CIBA-GEIGY Corporation is submitting pursuant to the TSCA Section 8(e) Compliance Audit Program and CAP Agreement number 8E CAP-0024. The information being submitted is not considered Confidential Business Information. We are submitting the following information, as required by the CAP Agreement:

Company Name: CIBA-GEIGY Corporation
444 Saw Mill River Road
Ardsley, New York 10502-2699

Attention: Mr. Anthony Di Battista
Manager, Regulatory Affairs & Toxic Substances
Compliance
Telephone (914) 479-2776

Tested Chemical: Anthra{9,1,2-cde}benzo{rst}pentaphene-5,10-dione, 16-nitro-; Also identified by a sample code number "DETO 0-09";

CAS No.: 128-60-9

Report Title: Mutagenicity Evaluation of DETO 0-09 (Report No. 20838, dated 1/22/78]

Summary: The test material was evaluated for genetic activity in Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 and Saccharrmyces cerevisiae strain D4 with and without the addition of mammalian metabolic activation preparations. In the absence of metabolic activation, the test material was positive in all strains except TA-1535 and D4. The results of the tests conducted on the test material with

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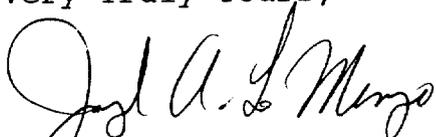
metabolic activation were positive in strains TA-1537, TA-1538, and TA-98. In strain TA-98, revertant frequency exceeded the positive controls both with and without metabolic activation. In a corroborative study, the test material was positive (induced reversion mutations) in all strains with and without metabolic activation, and in most cases, exceeded the corresponding positive controls. These studies are submitted based on the strong positive results and the potential for human exposure.

Category: Unit II.B.2.b

Prior Reporting: Not Applicable

Please call the undersigned at telephone number (919) 632-2889 if you have any questions about this submittal.

Very Truly Yours,



Joseph A. LoMenzo, Ph.D.
Product Stewardship Director
Textile Products Division

Enclosures

2 copies of this letter
3 copies of the study

cc: A. Di Battista

Contains NO CBI

CG Name: Cibanone Black DM Db1 Pst
CG No: 44/121996/100/0
TRC Name: Vat Black DD Db1 Pst
TRC No: 7185 00

RECEIVED BY [unclear] MAR - 9 1978

MUTAGENICITY EVALUATION

OF

DETO 0-09

Vat Black DD cake

P 87480

FINAL REPORT

TR 77-855 (b)

11/3/77 Ba. 24/77

SUBMITTED TO

DYES ENVIRONMENTAL AND TOXICOLOGY ORGANIZATION, INC.
1075 CENTRAL PARK AVENUE
SCARSDALE, NEW YORK 10583

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20838

JANUARY, 1978



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SPONSOR: Dyes Environmental and Toxicology Organization, Inc.

MATERIAL: Deto 0-09

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: November 30, 1977

2. Description: Black powder

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN	4 μ moles
Glucose-6-phosphate	5 μ moles
Sodium phosphate (dibasic)	100 μ moles
MgCl ₂	8 μ moles
KCl	33 μ moles
Homogenate fraction	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.



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2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b

^a Concentrations given in Results Section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.



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3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells: Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

4. RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: DETO 0-09
 B. SOLVENT: DMSO
 C. TEST INITIATION DATE: 12/08/77
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE											
			TA-1535		TA-1537		TA-1538		TA-98		TA-100		D4*	
NOACTIVATION	1	2	1	2	1	2	1	2	1	2	1	2	1	2
SOLVENT CONTROL	---	---	---	---	---	---	---	---	---	---	---	---	---	---
POSITIVE CONTROL**	---	---	---	---	---	---	---	---	---	---	---	---	---	---
TEST COMPOUND	0.10000 UG	18	25	34	54	175	24	34	54	175	24	34	54	175
	1.00000 UG	347	494	>1000	772	794	598	>1000	772	794	598	>1000	772	794
	10.00000 UG	32	56	67	273	181	35	67	273	181	35	67	273	181
	100.00000 UG	20	230	399	856	442	33	399	856	442	33	399	856	442
	500.00000 UG	26	437	840	>1000	498	28	840	>1000	498	28	840	>1000	498
	500.00000 UG	0	0	0	631	0	50	0	631	0	50	0	631	0
	500.00000 UG	0	0	0	0	0	42	0	0	0	42	0	0	0
ACTIVATION	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SOLVENT CONTROL	---	---	---	---	---	---	---	---	---	---	---	---	---	---
POSITIVE CONTROL***	---	---	---	---	---	---	---	---	---	---	---	---	---	---
TEST COMPOUND	0.10000 UG	21	14	26	36	224	52	26	36	224	52	26	36	224
	1.00000 UG	189	203	544	661	674	83	544	661	674	83	544	661	674
	10.00000 UG	18	9	29	40	248	36	29	40	248	36	29	40	248
	100.00000 UG	30	12	33	107	279	44	33	107	279	44	33	107	279
	500.00000 UG	24	117	243	678	388	25	243	678	388	25	243	678	388
	500.00000 UG	55	0	105	>1000	237	34	105	>1000	237	34	105	>1000	237
	500.00000 UG	0	0	0	268	0	47	0	268	0	47	0	268	0

* TRY+ CONVERTANTS PER PLATE

** TA-1535 MNNG 10 UG/PLATE
 TA-1537 GM 10 UG/PLATE
 TA-1538 NF 100 UG/PLATE
 TA-98 NF 100 UG/PLATE
 TA-100 MNNG 10 UG/PLATE
 D4 MNNG 10 UG/PLATE
 SOLVENT DMSO 50 ML/PLATE

*** TA-1535 ANTH 100 UG/PLATE
 TA-1537 AMU 100 UG/PLATE
 TA-1538 AAF 100 UG/PLATE
 TA-98 AAF 100 UG/PLATE
 TA-100 ANTH 100 UG/PLATE
 D4 DMNA 100 UG/PLATE
 SOLVENT DMSO 2.5 %/PLATE

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity Test Results

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.1 µg to 500 µg per plate. The compound was toxic to all the strains except D4 at 100 and 500 µg per plate.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were positive with all the strains except TA-1535 and D4. A dose-related increase in revertant frequency was also observed with TA-1537, TA-1538, TA-98 and TA-100.

C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were positive with the strains TA-1537, TA-1538 and TA-98. The compound exhibited genetic activity with TA-1537 at 10 µg dose level and the next two higher doses were toxic. The strain, TA-98, exhibited a dose-related increase in revertant frequency.



5. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

D. Conclusions

The test compound, Deto 0-09, exhibited genetic activity with TA-1537, TA-1538, and TA-98 strains in activation and nonactivation assays and with TA-100 in nonactivation assays conducted in this evaluation and is considered as mutagenic under these test conditions.

Submitted by:

D.R. Jagannath 1-13-78
D.R. Jagannath, Ph.D. Date
Section Chief
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Department of Molecular
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Reviewed by:

David J. Brusick 4/3/78
David J. Brusick, Ph.D. Date
Director
Department of Molecular
Toxicology



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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.



6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a



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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.



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STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.



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Triage of 8(e) Submissions

Date sent to triage: 2/5/96

NON-CAP

CAP

Submission number: 12282A

TSCA Inventory:

Y

N

D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

SEN

w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX

CTOX

EPI

RTOX

GTOX

STOX/ONCO

CTOX/ONCO

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

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pages 1,2

pages 1,2, tabs

Notes: 2-sided.

Contractor reviewer: LPS

Date: 5/17/95

CECATS/TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # SEHO-1192-12282 SEQ. A

TYPE: INT SUPP FLWP

SUBMITTER NAME: Ciba-Geigy Corporation

INFORMATION REQUESTED: FLWP DATE: _____
 0501 NO INFO REQUESTED
 0502 INFO REQUESTED (TECH)
 0503 INFO REQUESTED (VOL ACTIONS)
 0504 INFO REQUESTED (REPORTING RATIONALE)
 DISPOSITION:
 0639 REFER TO CHEMICAL SCREENING
 0678 CAP NOTICE

VOLUNTARY ACTIONS:
 0401 NO ACTION REPORTED
 0402 STUDIES PLANNED (TIMED WAY)
 0403 NOTIFICATION IN WORKER'S MIDDLE
 0404 LABEL/MSDS CHANGES
 0405 PROCESS/AND/IMP. CHANGES
 0406 APPAUSE DISCONTINUED
 0407 PRODUCTION DISCONTINUED
 0408 CONFIDENTIAL

SUB. DATE: 10/09/92 OTH DATE: 11/02/92 CSRAD DATE: 04/04/95

CHEMICAL NAME:
DETO 0-09
Cibanone Black DM Dbl Pst
Vat Black DD Dbl Pst

CASE
128-60-9
 "
 "

INFORMATION TYPE:	P.F.C.	INFORMATION TYPE:	P.F.C.	INFORMATION TYPE:	P.F.C.
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEMPHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 BODWAQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/ITERATO (HUMAN)	01 02 04	0221 ENV. OCCUREL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/ITERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQEST DELAY	01 02 04	0248 PRODUSE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	0251 MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0259 OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0239 METABPHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METABPHARMACO (HUMAN)	01 02 04		

<u>TRACED BY:</u>	<u>NON-CBI INVENTORY</u>	<u>ONGOING REVIEW</u>	<u>SPECIES</u>	<u>TOXICOLOGICAL CONCERN:</u>	<u>USE:</u>	<u>PRODUCTION:</u>
	<u>YES</u>	<u>YES (DROP/REFER)</u>	<u>In vitro</u>	<u>LOW</u>	<u>Dye</u>	
<u>CAS SR</u>	<u>NO</u>	<u>NO (CONTINUE)</u>		<u>MED</u>		
	<u>IN PROGRESS</u>	<u>REFER</u>		<u>HIGH</u>		

CONTACTS

4) ✓

8EHQ-92-12282: Rank - high.

Chemical: 16-nitroanthra[9,1,2-cde]benzo[*rst*]pentaphene-5,10-dione
(Vat Black DD cake Dbl Pst; Cibacron Black DM Dbl Pst; DETO 0-09;
CAS# 128-60-9).

Mutagenicity evaluation of DETO 0-09, Litton Bionetics, Inc.,
Kensington MD, dated January, 1978: Strongly positive for
gene mutations, with dose response, in Salmonella typhimurium
in strains TA98, TA1537 and TA1538 both without and with
metabolic activation, in strain TA100 without but not with
activation, and negative in strain TA1535 both without and
with metabolic activation.

Negative for DNA effects (gene conversion) in Saccharomyces
cerevisiae strain D4 both without and with metabolic
activation.

NOTE

Ranked high due to strong response in the
Salmonella/Ames assay