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The Bovine Corneal Opacity and Permeability (BCOP) Test Method − Validation Status and Appropriate Use

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NICEATM-ICCVAM Evaluation of BCOP

- Reviewed available data and information regarding the usefulness and limitations for assessing the ocular hazard potential of chemicals and products

- Determined validation status
  - Accuracy: sensitivity and specificity
  - Reproducibility for identifying ocular corrosives/severe irritants vs. all other hazard categories
  - Scope of substances tested
  - Availability of a standardized test method protocol

- Independent international scientific peer review panel

Overview of BCOP

- Minimum of three bovine corneas are treated with the test substance
  - 20% (w/v) suspension in 0.9% sodium chloride solution as described for solid nonsurfactant test substances
- Minimum of three bovine corneas are treated with the positive control
  - 20% (w/v) imidazole in 0.9% sodium chloride solution as suggested for solid test substances
- Minimum of three bovine corneas are treated with the solvent/vehicle control
  - 0.9% sodium chloride
- Time of exposure for solids: 4 hours
- Endpoints measured: Corneal opacity and permeability

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Validation Database

- 211 substances representing a wide range of chemical classes and product categories

- Sufficient *in vivo* data to assign an ocular irritancy classification according to the EPA and GHS classification systems

  - EPA : 187 substances

  - GHS: 187 substances

- Reproducibility data from three validation studies
  - Balls et al. (1995): 5 laboratories
  - Gautheron et al. (1994): 11-12 laboratories
  - Southee (1998): 3 laboratories

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Chemical Classes Tested in BCOP

Chemical Class	# of Substances	Chemical Class	# of Substances
Acyl halide	3	Inorganic Salt	6
Alcohol	22	Ketone	12
Aldehyde	1	Lactone	3
Alkali	3	Nitrile compound	1
Aluminum compound	1	Nitro compound	2
Amide	2	Oil	1
Amidine	6	Onium compound	12
Amine	10	Organic Salt	3
Amino Acid	4	Organic Sulfur compound	5
Boron compound	1	Organophosphate	1
Carboxylic Acid	17	Organiosilicon compound	1
Ester	12	Phenol	1
Ether/Polyether	9	Polycyclic compound	3
Formulation	69	Terpene	1
Heterocyclic compound	12	Wax	1
Hydrocarbon	18		
Imide	2		

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Product Categories Tested in BCOP

Product Category	# of Substances	Product Category	# of Substances
Adhesive	1	Fertilizer	1
Agricultural chemical	2	Flame retardant	1
Antifreeze agent	1	Flavor ingredient	3
Antimicrobial cleaning product	66	Food additive	1
Bactericide/Fungicide/Disinfectant/Germicide	11	Herbicide	3
Beverage	1	Insect repellant	8
Bleach	3	Lubricant/lubricant additive	6
Chelating agent	2	Paint, lacquer, varnish (component)	1
Chemical/synthetic intermediate	28	Pesticide	8
Cleaner	15	Petroleum product	16
Cleanser (personal care)	13	Photographic chemical/developing agent	2
Coupling agent	1	Plant growth regulator	2
Cutting fluid	2	Plasticizer	4
Degreaser	1`	Preservative	2
Dessicant	1	Reagent	5
Detergent	11	Shampoo (hair)	14
Drug/Pharmaceutical	17	Soap	3
Dry cleaning preparation	1	Solvent	34
Dye, in manufacture of	3	Surfactant	39
Emulsifier	1	Anionic surfactant	3
Etching and/or electroplating	2	Cationic surfactant	6
Explosive	1	Nonionic surfactant	5
Fabric softener	1	Thermometer fluid	1

Decision Criteria Proposed to Classify BCOP Data

	≥ 55.1 for Severe
Nonlabeled[2]	0 - 3
Mild Irritant[3]	3.1 - 25
Moderate Irritant[4]	25.1 - 55
Severe Irritant/Corrosive[1]	≥ 55.1

[1]EPA Category I; GHS Category 1
[2]EPA Category IV; GHS Not Labeled
[3]EPA Category III; GHS Category 2B
[4]EPA Category II; GHS Category 2A

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BCOP Test Method Accuracy: Ocular Corrosives and Severe Irritants[1]

Severe ≥55.1	No.	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA	187	79	147/187	84	53/63	76	95/124	24	29/124	16	10/63
GHS	187	79	148/187	85	55/65	76	93/122	24	29/122	15	10/65

[1] EPA = Cat I vs. Cat II/III/IV, GHS = Cat 1 vs. Cat 2A/2B/NC

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BCOP Interlaboratory Reproducibility

- Extent of agreement between testing laboratories when identifying ocular corrosives and severe irritants

% Agreement Among 19-20 Labs	EPA	GHS
100% (all substances)	71% (90/127)	72% (91/127)
≥73% (all substances)	89% 113/127)	88% (112/127)
100% (only corrosive/severe irritant)	75% (15/20)	78% (21/27)
≥75% (only corrosive/severe irritant)	90% (18/20)	93% (25/27)

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ICCVAM Test Method Recommendations for BCOP: Usefulness and Limitations
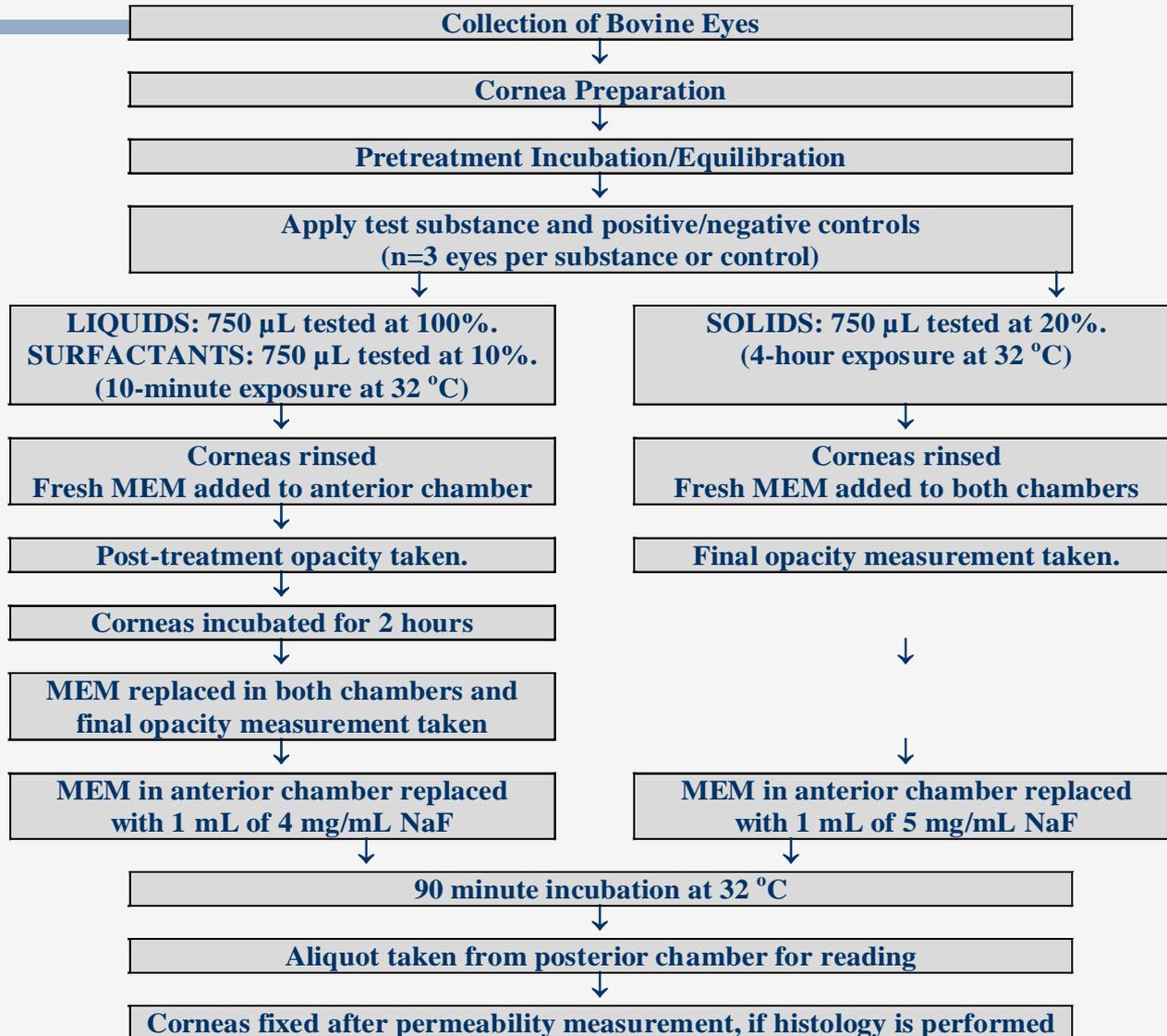
Usefulness

- Can be used for identification of ocular corrosives and severe irritants (EPA Category I, GHS Category 1) in appropriate circumstances and with certain limitations

Limitations

- Alcohols: 53% (8/15) to 56% (9/16) false positives[1]

- Ketones: 40% (4/10) false positives

- Solids: 42% (5/12) to 50% (5/10) false negatives

[1] False negative rates ranged from 67% (2/3) to 100% (2/2)

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ICCVAM-Recommended BCOP Protocol[1]

```
Collection of Bovine Eyes
        ↓
Cornea Preparation
        ↓
Pretreatment Incubation/Equilibration
        ↓
Apply test substance and positive/negative controls
(n=3 eyes per substance or control)
```

LIQUIDS: 750 µL tested at 100%. SURFACTANTS: 750 µL tested at 10%. (10-minute exposure at 32 °C)	SOLIDS: 750 µL tested at 20%. (4-hour exposure at 32 °C)
Corneas rinsed Fresh MEM added to anterior chamber	Corneas rinsed Fresh MEM added to both chambers
Post-treatment opacity taken.	Final opacity measurement taken.
Corneas incubated for 2 hours	
MEM replaced in both chambers and final opacity measurement taken	
MEM in anterior chamber replaced with 1 mL of 4 mg/mL NaF	MEM in anterior chamber replaced with 1 mL of 5 mg/mL NaF

```
90 minute incubation at 32 °C
        ↓
Aliquot taken from posterior chamber for reading
        ↓
Corneas fixed after permeability measurement, if histology is performed
```

[1]Available at http://iccvam.niehs.nih.gov/docs/ocutox_docs/OTeval/appD.pdf

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ICCVAM Test Method Recommendations: Future Studies

- Additional optimization studies/evaluations to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants

- Further evaluate the usefulness and limitations of using histopathological evaluation of the corneal tissue when BCOP is conducted

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BCOP International Acceptance

- **OECD TG 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants**
  - Adopted September 7, 2009
  - Available at: http://www.oecd-ilibrary.org/environment/test-no-437-bovine-corneal-opacity-and-permeability-test-method-for-identifying-ocular-corrosives-and-severe-irritants_9789264076303-en
  - Based on ICCVAM-recommended BCOP protocol
  - Expected to result in broader use of BCOP, which will further reduce and refine animal use for ocular safety assessments while ensuring human safety

2010 ICCVAM Evaluation of BCOP

- In 2010, ICCVAM also evaluated BCOP for identifying nonsevere irritants and substances not labeled as irritants

- However, ICCVAM concluded that BCOP is **not** recommended as a screening test to distinguish substances not labeled as irritants from all other hazard categories

- ICCVAM also concluded that BCOP is **not** recommended to identify moderate and mild ocular irritants as defined by the EPA and GHS classification systems

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