Empagliflozin Genotoxic Impurity Assessment Report

This dossier-style report follows the Empagliflozin genotoxic impurity format and includes formal genotoxicity and theoretical purge-factor summary tables.

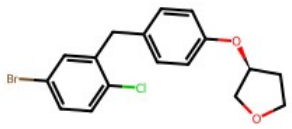
Assessment Basis

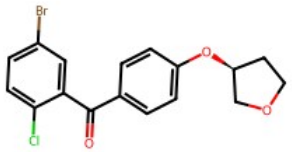
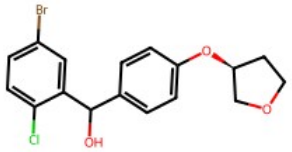
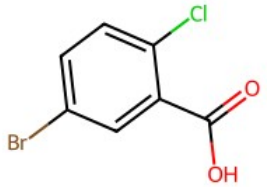
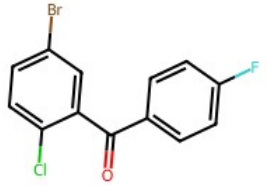
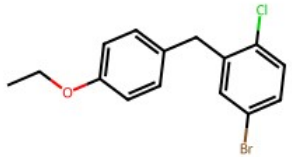
Reference dataset: public Ames mutagenicity reference set | Validation rows: 2352 | Positive threshold: 0.70 | Equivocal threshold: 0.40

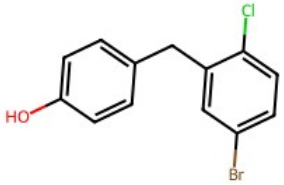
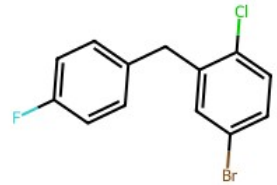
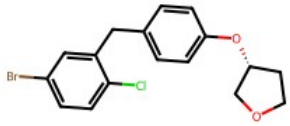
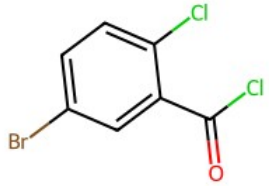
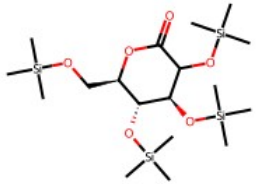
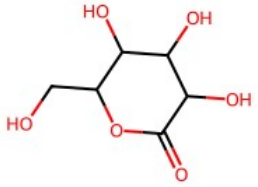
Table 1. ICH M7 Classification Reference

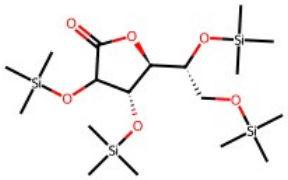
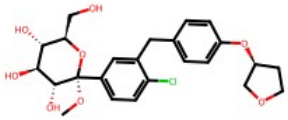
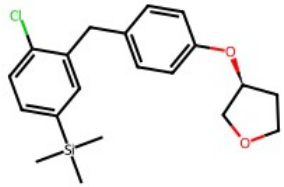
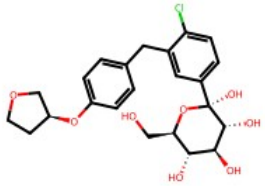
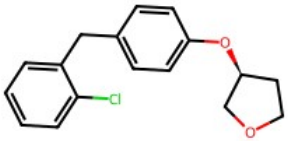
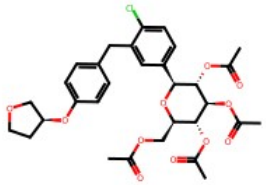
Class	Definition	Proposed Action For Control
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit.
2	Known mutagens with unknown carcinogenic potential	Control at or below acceptable limits using the appropriate TTC or compound-specific limit.
3	Alerting structure unrelated to the drug substance and no mutagenicity data	Control at or below acceptable limits or perform bacterial mutagenicity testing.
4	Alert shared with the drug substance or API-related compounds shown to be non-mutagenic	Treat as non-mutagenic impurity with documented scientific rationale.
5	No structural alert, or sufficient data showing lack of mutagenicity/carcinogenicity	Treat as non-mutagenic impurity.

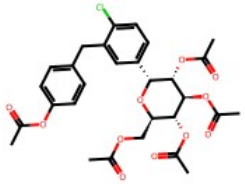
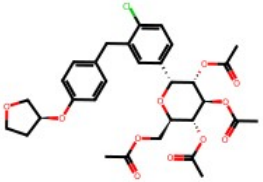
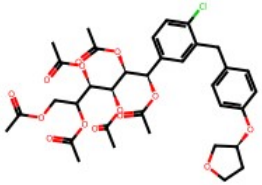
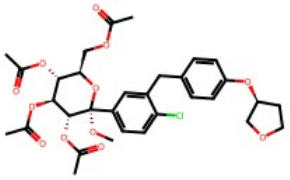
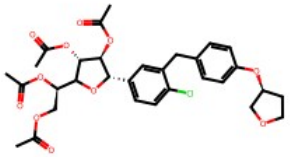
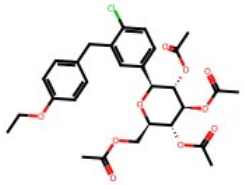
Table 2. Results For Genotoxic Impurities

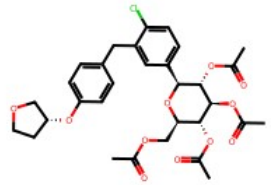
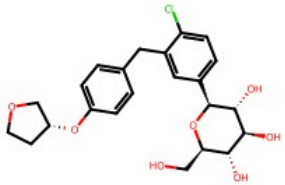
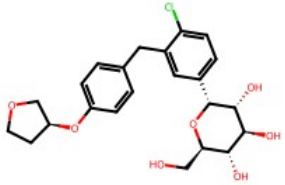
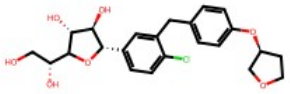
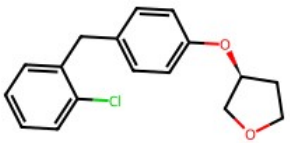
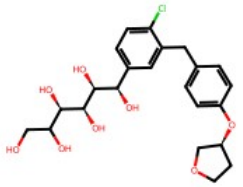
Name / Compound ID	Structure	Expert Rule-Based System	Statistical-Based System	ICH M7 Class
BCPT		Negative	Equivocal	Class 5

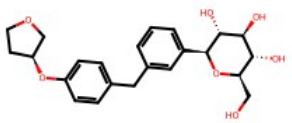
BCTPM		Negative	Equivocal	Class 5
EG4-Br-07		Negative	Equivocal	Class 5
EG1-Br		Negative	Negative	Class 5
EG2-Br		Negative	Negative	Class 5
EG4-Br-6		Negative	Negative	Class 5
EG4-Br-10		Negative	Equivocal	Class 5

EG4-Br-12		Negative	Negative	Class 5
EG4-Br-13		Negative	Negative	Class 5
EG4-Br-05		Negative	Equivocal	Class 5
(EG1-Br Acyl chloride)		Positive	Negative	Class 3
(EGM-TMS		Negative	Equivocal	Class 5
(D -gluconolactone)		Negative	Negative	Class 5

EGA five cyclic heterocycle		Negative	Equivocal	Class 5
CTBHM		Negative	Equivocal	Class 5
(EG-10)		Negative	Equivocal	Class 5
(EG-07)		Negative	Equivocal	Class 5
EG4-Br-08 (impurity D)		Negative	Equivocal	Class 5
(AC-EPG)		Negative	Equivocal	Class 5

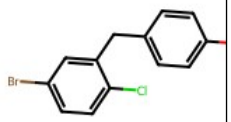
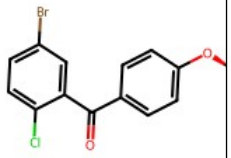
(AC-EG-04)		Negative	Equivocal	Class 5
AC-EG-02		Negative	Equivocal	Class 5
(AC-EG-09)		Negative	Equivocal	Class 5
(AC-EG6)		Negative	Equivocal	Class 5
(AC-EG-01)		Negative	Equivocal	Class 5
ACDG		Negative	Equivocal	Class 5

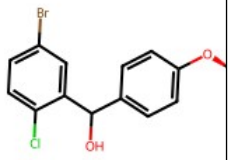
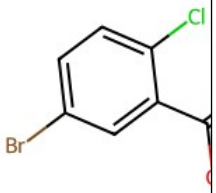
(AC-EG-05)		Negative	Equivocal	Class 5
(Impurity A)		Negative	Equivocal	Class 5
(Impurity B)		Negative	Equivocal	Class 5
(Impurity C)		Negative	Equivocal	Class 5
(Impurity D)		Negative	Equivocal	Class 5
(Impurity E)		Negative	Equivocal	Class 5

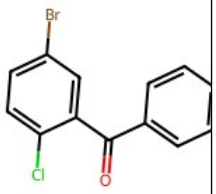
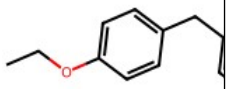
(Impurity F)		Negative	Equivocal	Class 5

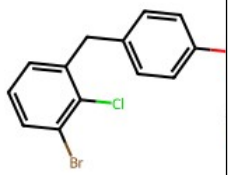
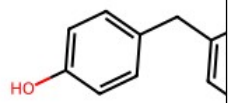
The table format mirrors the Empagliflozin dossier style. The expert and statistical columns are produced from the local alert library and local QSAR ensemble,.

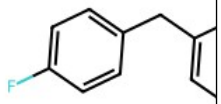
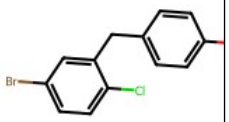
Table 3. Theoretical Purge Factor Assessment

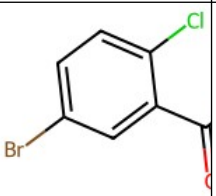
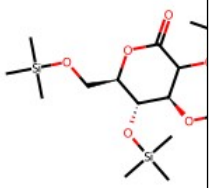
Name / Compound ID	Structure	Hazard Alert Status	ICH M7 Class	Total Purge Factor	Purge Assessment	Control Strategy
BCPT		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
BCTPM		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

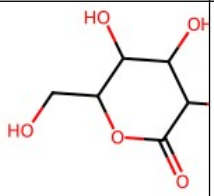
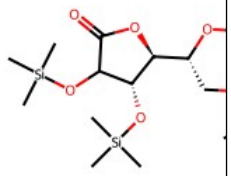
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG4-Br-07		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG1-Br		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

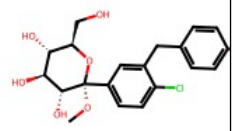
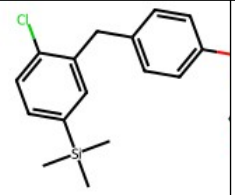
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG2-Br		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG4-Br-6		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

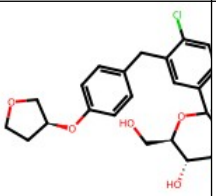
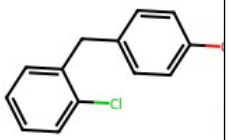
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG4-Br-10		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG4-Br-12		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

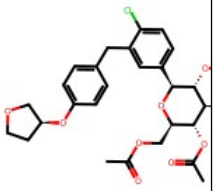
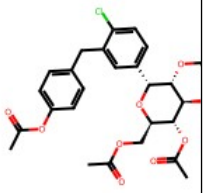
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG4-Br-13		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG4-Br-05		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

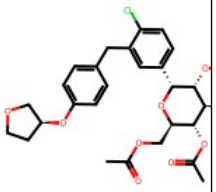
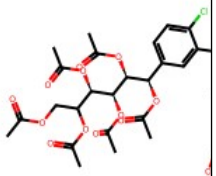
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(EG1-Br Acyl chloride)		alert_present	Class 3	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(EGM-TMS		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

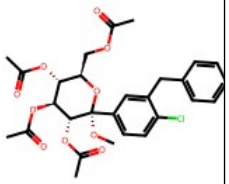
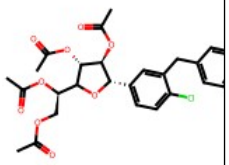
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(D -gluconolactone)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EGA five cyclic heterocycle		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

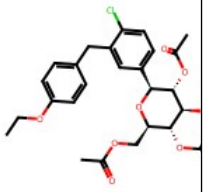
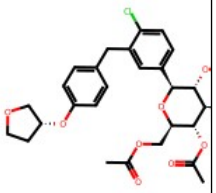
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
CTBHM		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(EG-10)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

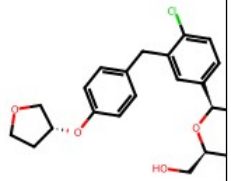
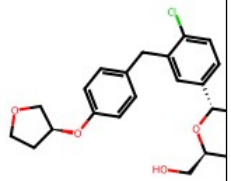
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(EG-07)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
EG4-Br-08 (impurity D)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

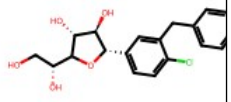
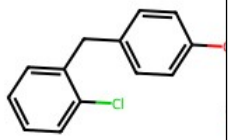
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(AC-EPG)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(AC-EG-04)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

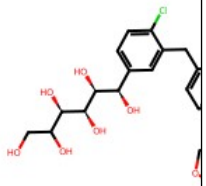
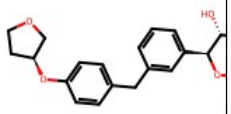
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
AC-EG-02		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(AC-EG-09)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(AC-EG6)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(AC-EG-01)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
ACDG		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(AC-EG-05)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

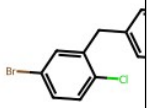
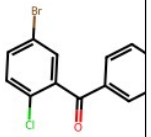
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(Impurity A)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(Impurity B)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

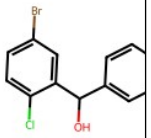
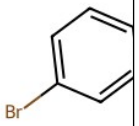
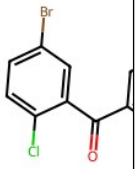
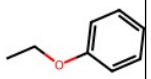
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(Impurity C)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(Impurity D)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

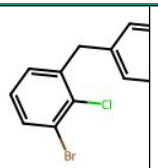
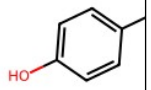
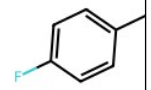
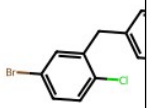
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(Impurity E)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid, ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.
(Impurity F)		no_structural_alert	Class 5	not_provided	not_provided	Actual manufacturing process uses potassium hydroxide, hydrochloric acid,

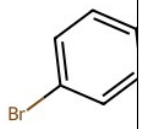
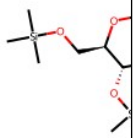
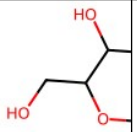
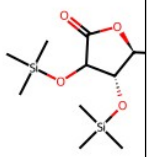
						ethanol, purified water, and ethyl acetate. No nitrite source or nitrosating reagent is listed; nitroso risk control depends on exclusion of nitrite contamination during acidic aqueous processing.

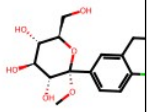
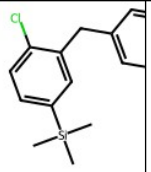
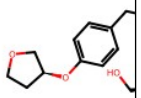
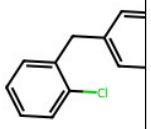
Table 4. Nitroso / Nitrosamine Impurity Possibility Assessment

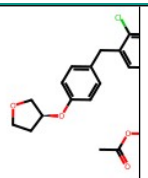
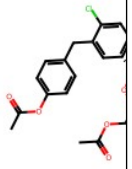
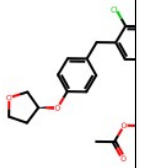
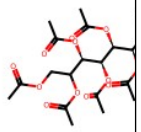
Name / Compound ID	Structure	Nitrosatable Precursor	Nitrite Source	Acidic Quench	Nitrosating Reagent	Recovered / Recycled Stream	Wet Process	Possibility	Basis
BCPT		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
BCTPM		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

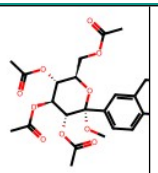
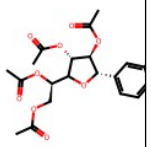
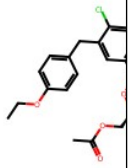
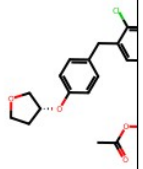
EG4-Br-07		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EG1-Br		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EG2-Br		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EG4-Br-6		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

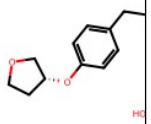
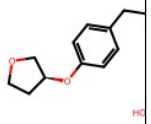
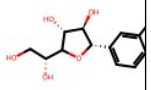
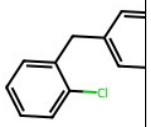
EG4-Br-10		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EG4-Br-12		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EG4-Br-13		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EG4-Br-05		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

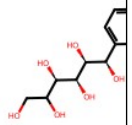
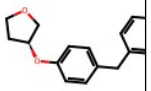
(EG1-Br Acyl chloride)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(EGM-TMS		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(D -gluconolactone)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EGA five cyclic heterocycle		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

CTBHM		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(EG-10)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(EG-07)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
EG4-Br-08 (impurity D)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

(AC-EPG)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(AC-EG-04)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
AC-EG-02		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(AC-EG-09)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

(AC-EG6)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(AC-EG-01)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
ACDG		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(AC-EG-05)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

(Impurity A)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(Impurity B)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(Impurity C)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(Impurity D)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

(Impurity E)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.
(Impurity F)		No	No	Yes	No	No	Yes	no_structural_nitrosation_concern	No obvious nitrosatable amine precursor was detected by the current structural rules.

Literature Integration

Ich M7 Workflow: EUROTOX 2016 GTI workflow: complementary expert-rule and statistical methodologies with class 3/4/5 reasoning.

Muta Comparison: Bakhtyari et al. 2013 comparison of mutagenicity models: statistical QSAR performed better overall and applicability domain matters.

Nitrosamine Lessons: EMA lessons learnt for nitrosamines: assess nitrite sources, amines, recovered materials, solvent systems, and process conditions.

Nitrosamine Purge: Borths et al. nitrosamine reactivity and purge: use chemistry and purge opportunities, not just structure alerting.

Model Validation

Model	Type	Representation	Accuracy	Balanced Accuracy	Sensitivity	Specificity	F1	ROC AUC
rf_morgan	random_forest	morgan_2048_r2	0.713	0.688	0.509	0.866	0.603	0.776

lr_rdkit	logistic_regression	rdkit_physchem	0.621	0.568	0.200	0.935	0.311	0.598