



# Preclinical Models in Antiemetic Research: Mechanistic Pathways, Experimental Protocols, and Translational Applications *Experimental Emesis Models in Drug Development: Comparative Protocols and Mechanistic Rationale*

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## ABSTRACT :

**Background:** The emetic reflex is a complex neurophysiological process regulated by both central and peripheral mechanisms. Despite the availability of antiemetic drugs, current therapies often show limited efficacy across diverse clinical conditions, such as chemotherapy-induced nausea and vomiting (CINV), radiation-induced emesis, and motion sickness. This highlights the necessity for robust preclinical models that can accurately predict clinical efficacy and dissect mechanistic pathways.

**Objective:** This review aims to systematically classify, analyze, and compare a wide array of preclinical models used in the evaluation of antiemetic activity. The focus is on their mechanistic underpinnings, experimental protocols, translatability, and predictive relevance for drug development.

**Methods:** An extensive literature survey was conducted using PubMed, Scopus, and Web of Science databases (2020–2024), covering animal models (emetic and non-emetic species), surrogate behaviors (kaolin pica, conditioned taste aversion), in vitro assays (receptor binding, ileal contraction), and human provocation models (ippecac test, virtual-reality induced nausea). Each model was evaluated for its relevance to specific emetogenic pathways and classes of antiemetic agents.

**Results:** The review classifies antiemetic models into five major categories:

*In Vivo Emetic Models* (e.g., ferrets, dogs, cats, monkeys, shrews, chicks)

*Non-Emetic Surrogate Models* (rats, mice, zebrafish larvae)

*Behavioral and Neurophysiological Markers* (pica, gaping, facial grooming)

*In Vitro and Ex Vivo Assays* (5-HT<sub>3</sub>, D<sub>2</sub>, NK<sub>1</sub> receptor screens)

*Human Provocation Models* (ippecac-induced, fMRI-guided, VR-induced nausea)

Each model's experimental protocol, validation parameters, and suitability for specific antiemetic drug classes (e.g., serotonin antagonists, NK<sub>1</sub> blockers, corticosteroids, herbal agents) are critically analyzed. A *model selection matrix* is proposed for guiding rational use based on mechanistic and translational needs.

**Conclusion:** A deep understanding of model-specific emetic pathways and response biomarkers is crucial for selecting appropriate experimental platforms. This review provides a valuable reference framework for researchers and regulatory agencies in optimizing preclinical antiemetic studies. Future advancements may include AI-integrated emetic prediction, organ-on-chip emesis models, and gut–brain axis simulations.

**Keywords:** Antiemetic activity; Preclinical models; Emetic reflex; Translational pharmacology; Cisplatin-induced vomiting; Surrogate nausea models; Neurotransmitter receptors; Ipecac test; Kaolin pica; Drug development

## 1. Introduction

Nausea and vomiting are complex protective reflexes involving a coordinated interplay between the gastrointestinal tract and central nervous system. They pose a significant burden in clinical contexts such as chemotherapy, surgery, motion sickness, and infection [1]. Among these, chemotherapy-

induced nausea and vomiting (CINV) is one of the most distressing side effects, affecting approximately 57–80% of patients receiving moderate to highly emetogenic regimens and significantly impairing quality of life and treatment adherence [2,3]. Despite advances in antiemetic therapy—especially with the introduction of 5-HT<sub>3</sub> and NK<sub>1</sub> receptor antagonists—control of delayed and anticipatory nausea remains suboptimal. Real-world studies report that complete control rates of CINV rarely exceed 60%, even with guideline-based prophylaxis, with many patients experiencing breakthrough or refractory symptoms [4,5]. This highlights a pressing need for better predictive models and improved translational tools in antiemetic drug development.

The emetic reflex is mediated through the dorsal vagal complex (DVC)—including the area postrema, nucleus tractus solitarius, and dorsal motor nucleus of the vagus—and receives input from chemoreceptor, vestibular, and vagal afferents [6]. Neurotransmitters integral to this pathway include serotonin (5-HT), substance P, dopamine, histamine, and acetylcholine, which have become key pharmacological targets in antiemetic therapy [7,8].

Current preclinical and clinical antiemetic assessments rely on a diverse set of experimental models: emetic models (e.g., cisplatin-induced vomiting in *Suncus murinus* and ferrets), surrogate models (e.g., kaolin-induced pica in rats), motion-induced emesis, radiation-induced emesis, in vitro receptor assays, and human-provocation tests [9–12]. However, fragmented protocols, lack of standardization, and varying translational relevance complicate cross-study comparisons and clinical extrapolation.

This review aims to:

1. Map and categorize all relevant *in vivo*, *ex vivo*, *in vitro*, and *clinical* models currently employed in antiemetic research;
2. Provide detailed, standardized experimental protocols (agent, dose, timing, endpoints) to enhance reproducibility;
3. Evaluate models comparatively based on translational utility, cost, and ethical considerations;
4. Highlight emerging paradigms, including the gut–brain axis, microbiome interactions, and AI-based behavioral analytics, showcasing future research directions.

By integrating mechanistic understanding with practical protocols across species and model types, this comprehensive review will serve as a reference toolkit for researchers, drug developers, and clinicians aiming to improve the translational development of next-generation antiemetic therapies.

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## 2. Mechanisms of Emesis: Central and Peripheral Pathways

Nausea and vomiting result from a coordinated network involving *central integration*, *gut–brain signaling*, and *motor outputs*—with the dorsal vagal complex (DVC) at the core.

### 2.1 The Dorsal Vagal Complex (DVC)

The DVC comprises the *area postrema (AP)*, *nucleus tractus solitarius (NTS)*, and *dorsal motor nucleus of the vagus (DMV)*. The AP—also known as the chemoreceptor trigger zone—is positioned on the floor of the fourth ventricle and is uniquely permeable, detecting blood-borne emetogens (chemotherapeutics, toxins) and transmitting signals to the NTS and DMV to initiate emesis [13,14,15].

Lesion studies confirm the AP's pivotal role in drug- and toxin-induced vomiting, although vestibular and vagal afferent stimulation can bypass it [13]. The NTS integrates inputs from the AP, vagus, vestibular system, and higher centers, while the DMV orchestrates autonomic motor output for vomiting [16,17].

### 2.2 Peripheral Afferents and the Gut–Brain Axis

Enterochromaffin cells (ECCs) in the gut mucosa detect toxins and release serotonin, which activates 5-HT<sub>3</sub> receptors on vagal afferents—transmitting signals to the DVC. Recent circuit-mapping studies have identified a gut-to-brain axis involving Htr3a<sup>+</sup> vagal neurons and Tac1<sup>+</sup> neurons in the DVC, which modulate both defensive vomiting behaviors and conditioned taste aversion [18].

### 2.3 Central Pattern Generators and Motor Response Coordination

Once signals converge in the DVC, central pattern generators (e.g., in the Böttinger complex) coordinate the *motor sequence of vomiting*: upper stomach relaxation, retrograde contraction, retching, and expulsion—mediated via vagal and somatic pathways [19].

Somatic motor output includes phrenic activation of the diaphragm and contraction of abdominal muscles. Autonomic functions such as salivation, pallor, sweating, and cardiovascular changes are concomitantly controlled [20].

### 2.4 Neurotransmitter Receptors Involved

Central emetic circuits involve multiple receptors that are pharmacological targets for antiemetics:

- *5-HT<sub>3</sub>*: key for acute emesis, antagonized by ondansetron
- *NK<sub>1</sub> (Substance P)*: critical for delayed emesis
- *Dopamine D<sub>2</sub>*: CTZ-mediated emesis, suppressed by metoclopramide
- *M<sub>1</sub>, H<sub>1</sub>*: significant in motion sickness and vestibular inputs
- *Others*: CB<sub>1</sub>, GPRs, and neuroinflammatory mediators [13,14,16]

### 3. Classification of Antiemetic Models

This section presents an in-depth classification of experimental antiemetic models, organized by *species and mechanism*, with *mechanistic rationale*, *protocols*, and *translational value*.

#### 3.1. In Vivo Emetic Models (Emetic Species)

##### 3.1.1: Cisplatin-Induced Emesis in Ferrets (Detailed Version)

*Species Used*: Adult male ferrets (*Mustela putorius furo*), weighing 1–1.5 kg, 12–16 weeks old. Ferrets are among the few laboratory animals that possess a vomiting reflex, making them an ideal model for emesis research [21].

*Experimental Protocol*:

1. *Animal Acclimatization*:
  - Ferrets are housed individually in metabolic cages.
  - Acclimatized for 7–10 days in a temperature-controlled room ( $22 \pm 2^\circ\text{C}$ ) under a 12 h light/dark cycle [22].
2. *Induction of Emesis*:
  - Cisplatin is administered *intraperitoneally (i.p.)* at a dose of 5–10 mg/kg.
  - Emesis is observed in *two distinct phases*:
    - *Acute phase*: 0–8 hours post-dose.
    - *Delayed phase*: 24–72 hours post-dose [23].
3. *Observation & Data Collection*:
  - Animals are video-monitored or observed continuously during acute phase and intermittently during delayed phase.
  - *Emesis is defined as the act of retching followed by vomitus expulsion.*
  - Parameters recorded:
    - Latency to first vomit.
    - Total number of emetic episodes (retching and vomiting).
    - Behavioral changes (e.g., piloerection, reduced activity).
4. *Controls*:
  - Vehicle-treated ferrets (0.9% saline).
  - Positive controls: Ondansetron (5-HT<sub>3</sub> antagonist), aprepitant (NK<sub>1</sub> antagonist) [24].
5. *Test Compound Evaluation*:
  - Candidate antiemetics are administered *prophylactically* (30–60 min before cisplatin) and/or *therapeutically* after onset of emesis.
  - Effects are measured in both acute and delayed phases.

*Mechanism of Emesis*:

- *Cisplatin triggers the release of serotonin (5-HT)* from enterochromaffin cells in the GI tract.
- Serotonin activates *5-HT<sub>3</sub> receptors on vagal afferents*, which relay signals to:
  - The *nucleus tractus solitarius (NTS)*.
  - The *area postrema (AP)* of the brainstem [25].
- This dual pathway explains the *biphasic emesis*:
  - *Acute phase*: Serotonergic.
  - *Delayed phase*: NK<sub>1</sub> and substance P-mediated [26].

*Validation and Clinical Relevance*:

- This model closely *mimics human CINV*, both in timing and mechanistic basis [27].
- *5-HT<sub>3</sub> receptor antagonists* (e.g., ondansetron) reduce acute emesis.
- *NK<sub>1</sub> antagonists* (e.g., aprepitant) reduce delayed emesis [28].
- Combining both yields synergistic control, as in clinical guidelines.

*Advanced Utility*:

- Recent improvements include *telemetric models* with:

- Implanted transmitters.
- Continuous data logging of retching and heart rate patterns [29].
- Ferret models are also used for evaluating cannabinoid, steroidal, and herbal antiemetics.

### 3.1.2: Apomorphine-Induced Emesis in Dogs and Ferrets (A Central Dopaminergic Model for Evaluating D<sub>2</sub> Antagonists)

#### Species Used:

- Dogs (beagle breeds, 7–15 kg, both sexes) and
- Ferrets (1–1.5 kg), both of which possess a functional emetic reflex. They have centrally located D<sub>2</sub> receptors in the *area postrema* (AP), making them ideal for studying central emesis pathways [30].

#### Experimental Protocol:

1. *Animal Preparation:*
  - Fast animals overnight with free access to water.
  - Place in individual observation cages with a clear front for video recording.
  - Acclimatize to handling for 3 days prior.
2. *Induction of Emesis:*
  - Administer *Apomorphine*:
    - Dose: 0.25–1.0 mg/kg *subcutaneously* (s.c.) or *intravenously* (i.v.) [31].
    - Onset of vomiting typically within 5–15 minutes.
3. *Observation Parameters:*
  - Latency to first vomit.
  - Total number of retches and vomitus expulsions within 1 hour post-injection.
  - Any accompanying behavior (e.g., salivation, panting, tremors).
4. *Controls:*
  - Saline-injected animals (negative control).
  - Known D<sub>2</sub> antagonists (positive control), e.g., *Metoclopramide* (0.5–2 mg/kg, i.v.) or *Domperidone* (1–5 mg/kg, i.p.) [32].
5. *Assessment:*
  - Degree of emesis inhibition is compared to controls to assess antiemetic efficacy.
  - Evaluation done by trained observers blinded to treatment.

#### Mechanism of Action:

- Apomorphine is a *non-selective dopamine agonist*, acting *primarily on central D<sub>2</sub> receptors* in the *area postrema* (CTZ – chemoreceptor trigger zone).
- It does *not affect peripheral pathways* (like 5-HT<sub>3</sub> or NK<sub>1</sub>), making it specific for *central emesis* models [33].

#### Validation and Applications:

- This model is widely used to *screen central dopaminergic antagonists* like:
  - *Metoclopramide*
  - *Domperidone*
  - *Haloperidol*
- Especially valuable in evaluating *drug-induced nausea*, *Parkinson's medications*, or other CNS-acting agents [34].

#### Translational Utility:

- *Strengths:*
  - Reproducible.
  - Specific for *central emetic mechanisms*.
  - Quick onset of vomiting allows for short assay duration.
- *Limitations:*
  - *Not useful for delayed or peripheral emesis mechanisms*, such as chemotherapy or motion sickness [35].
  - Does *not mimic human chemotherapy-induced emesis*, which involves peripheral serotonin release and substance P.

### 3.1.3: Radiation-Induced Emesis in Ferrets and Monkeys (A Translational Model for Radiation Therapy–Associated Vomiting)

#### Species Used:

- Ferrets (*Mustela putorius furo*)

- *Monkeys*, especially *Rhesus macaques* (*Macaca mulatta*)  
Both species exhibit *well-defined emetic responses*, and have been extensively used in *space medicine*, *radiation safety*, and *oncology drug development* [36].

#### Experimental Protocol:

1. *Pre-Exposure Preparation:*
  - Acclimatize animals for 7 days in controlled environment ( $22 \pm 1^\circ\text{C}$ , 12 h light/dark cycle).
  - Fast for 6–8 hours prior to radiation exposure.
2. *Radiation Induction:*
  - Animals receive *whole-body or abdominal exposure* to  $\gamma$ -radiation using cobalt-60 or cesium-137 sources.
  - *Dose range:* 2–6 Gray (Gy), depending on species and intended latency profile [37].
3. *Observation Timeline:*
  - *Latency Period:* Onset of emesis typically occurs *1–4 hours post-exposure*, peaking between *4–10 hours*, and lasting up to *24–36 hours*.
  - In ferrets, the *emesis peak is earlier*, whereas in monkeys, the duration and frequency are prolonged and dose-dependent [38].
4. *Behavioral Monitoring:*
  - Continuous video monitoring post-irradiation.
  - Parameters:
    - Latency to first emetic episode.
    - Total vomiting and retching episodes.
    - Associated symptoms (lethargy, piloerection, salivation).
5. *Treatment Assessment:*
  - Antiemetic agents (e.g., *palonosetron*, *dexamethasone*, *aprepitant*) are administered:
    - *Prophylactically:* 30–60 min before exposure.
    - *Therapeutically:* 1–3 hours post-exposure.
  - Drugs are evaluated for their ability to *suppress emetic frequency and extend latency*.

#### Mechanism of Emesis:

- *Ionizing radiation* damages *gastrointestinal epithelial cells*, especially *enterochromaffin (EC) cells*, leading to:
  - Release of *serotonin (5-HT)*.
  - Activation of *5-HT<sub>3</sub> receptors* on vagal afferents.
  - Signal relay to *nucleus tractus solitarius (NTS)* and *area postrema (AP)* [39].
- In higher doses, *neurokinin-1 (NK<sub>1</sub>)* and *substance P* signaling contributes to the *prolonged delayed phase* [40].

#### Validation and Application:

- *Highly predictive of radiation-induced nausea and vomiting (RINV)* in human patients undergoing *radiotherapy or nuclear medicine diagnostics*.
- Ferret model shows clear *dose–response correlation*, reproducibility, and sensitivity to *5-HT<sub>3</sub>* and *NK<sub>1</sub>* antagonists [41].
- Monkey models have been used in *NASA and military radiation countermeasure studies* [42].

### 3.1.4: Motion and Copper Sulfate-Induced Emesis in Cats (Vestibular and Gastrointestinal Stimuli in Feline Emetic Research)

**Species Used:** Domestic cats (~3–5 kg), typically mixed-breed, with a functional emetic reflex sensitive to vestibular and gastrointestinal stimuli [43].

#### 1. Motion-Induced Emesis (Vestibular Model)

##### Protocol:

1. *Acclimatization:* House cats individually, free access to water, standard chow; acclimate for 7 days.
2. *Vestibular Stimulation:* Place cats in a *rotating chair* or on a *tilt-table* providing controlled oscillatory or linear movement (e.g., 0.2–0.4 Hz for 10–20 minutes).
3. *Observation:* Begin immediately and record vomiting, retching, salivation, and vocalizations.
4. *Data Collected:*
  - Latency to first sign of nausea (e.g., lip licking, pacing).
  - Number of vomits/retch episodes.
5. *Validation:* Administer *diphenhydramine* (1–2 mg/kg, *i.m.*) or *scopolamine* (0.01 mg/kg, *s.c.*) 30 minutes before stimulation. Compare to saline control [44].

**Mechanism and Suitability:** Provokes the *vestibulocerebellar–vestibular nuclei pathway*, leading to nausea/vomiting through *H<sub>1</sub>* and *M<sub>1</sub>* receptor activation, analogous to human motion sickness. High translational relevance for motion-sickness treatments [45].

## 2. Copper Sulfate–Induced Emesis (Gastrointestinal/Visceral Model)

### Protocol:

1. *Preparation:* Fast cats for 12 hours; free access to water.
2. *Administration:* Give *copper sulfate (CuSO<sub>4</sub>)* at 50 mg/kg via orogastric tube.
3. *Observation Period:* Monitor for 30–60 minutes.
4. *Endpoints:* Latency to first vomit, total number of vomits, frequency of retching.
5. *Controls:* Pre-treat with *ondansetron* (0.1 mg/kg, i.v.) or *metoclopramide* (0.5 mg/kg, i.v.) [46].

*Mechanism and Suitability:* CuSO<sub>4</sub> irritates the GI tract, directly activating *gut vagal afferents* and triggering central emetic centers via serotonin and prostaglandin pathways. Highly suitable for testing *peripheral-acting antiemetics* and evaluating gastrointestinal irritant-induced vomiting.

### Translational Value & Limitations

- *Strengths:*
  - Combines two distinct pathways relevant to human emesis (motion and visceral irritation).
  - Cats provide robust and easily observable vomiting behaviors.
- *Limitations:*
  - Ethical and cost considerations limit routine use.
  - Less sensitive to delayed-phase or chemotherapy-induced vomiting patterns.

## 3.1.5: Radiation- and Emetogen-Induced Emesis in Monkeys (A High-Fidelity Primate Model for Human-Like Vomiting Responses)

### Species Used:

- *Rhesus macaques (Macaca mulatta)*
- Occasionally *Cynomolgus monkeys (Macaca fascicularis)*  
These primates have a well-developed emetic reflex and *central–peripheral emetic circuitry* that closely mimics humans [47].

### A. Radiation-Induced Emesis

#### Protocol:

1. *Pre-conditioning & Acclimatization:*
  - 10–14 days of environmental conditioning (21 ± 2 °C; 12 h light–dark).
  - Fasted overnight with water access.
2. *Radiation Exposure:*
  - Whole-body or partial abdominal *gamma irradiation* via *Cobalt-60* source.
  - Dose: 2.5 to 5.0 Gy, standardized for emesis latency and safety thresholds.
3. *Monitoring & Observation:*
  - *Time to onset*, number of vomits, retches, signs of nausea (salivation, crouching).
  - Behavior tracked for 6–24 h post-exposure.
4. *Validation Drugs:*
  - *Palonosetron* (0.25 mg/kg), *dexamethasone* (2 mg/kg), *aprepitant* (NK<sub>1</sub> antagonist), administered *prophylactically* or *after emesis onset*.

#### Mechanism:

- Damage to *GI epithelial cells*, especially *enterochromaffin (EC) cells*, leads to:
  - Release of 5-HT (serotonin) → 5-HT<sub>3</sub> receptor activation on *vagal afferents*.
  - Signal relayed to *area postrema (AP)* and *nucleus tractus solitarius (NTS)*.
- Delayed phase involves *substance P* and *NK<sub>1</sub> receptors*, resembling *chemotherapy- or radiation-induced nausea and vomiting (RINV)* in humans [48].

### B. Emetogen-Induced Emesis in Monkeys

#### Protocol:

1. *Common emetogens:*
  - *Apomorphine* (0.05–0.3 mg/kg, s.c.) – Central D<sub>2</sub> receptor agonist.
  - *Cisplatin* (0.5–2.0 mg/kg, i.v.) – For modeling chemotherapy-induced emesis.

- *Copper sulfate* (25–50 mg/kg, oral) – Induces gastric irritation.
- 2. *Observation:*
  - Pre-treatment and post-treatment with antiemetics as above.
  - Latency, frequency, and behavioral indicators scored.

#### Advantages of Monkey Models:

- Highly predictive for *clinical antiemetic efficacy*, especially in *chemotherapy*, *space radiation*, and *neuroactive emetogens* [49].
- Allow monitoring of *pharmacokinetics*, *CNS activity*, and *subjective behavioral correlates*.
- Excellent for *bridge studies (preclinical to human)* in emesis pharmacology.

#### Limitations:

- *High ethical considerations and regulatory hurdles*.
- Require *specialized housing and radiation containment facilities*.
- *Cost-intensive*, used selectively in *late-stage preclinical studies* or *biodefense/space medicine*.

### 3.1.6: Retching-Based Emetic Model in Young Chicks (*Gallus gallus domesticus*) (A Reflexive Behavioral Model Without Vomiting Reflex)

#### Species Used:

- *Day-old to 3-day-old chicks* (*Gallus gallus*)
- Despite lacking a functional vomiting reflex, chicks exhibit *retching*, *gaping*, and *distress vocalizations*, which are reliable surrogate endpoints of emesis-like responses [51].

#### Protocol:

1. *Housing and Conditioning:*
  - Maintain in brooder boxes (32–35 °C) with 12-h light/dark cycle.
  - Access to water and starter feed ad libitum.
  - Acclimatize chicks for 24 hours before experiment.
2. *Drug Administration:*
  - *Emetogens:*
    - *Copper sulfate* ( $\text{CuSO}_4$ , 50–100 mg/kg) orally via gavage.
    - *Lithium chloride* ( $\text{LiCl}$ , 150–200 mg/kg, *i.p.*)
    - *Cisplatin* (5–10 mg/kg, *i.p.*) for delayed effects.
  - *Test antiemetics:*
    - Administer agents like *ondansetron* (0.5–1 mg/kg, *i.p.*), *aprepitant*, or *scopolamine* 30 min prior.
3. *Observation Parameters:*
  - Duration: Monitor for 60 minutes.
  - *Endpoints:*
    - Number of *retching episodes* (mouth gaping, head extension).
    - Associated behaviors: decreased locomotion, vocalization, postural freezing.
  - Optional: Video-tracking for behavioral scoring.

#### Mechanistic Relevance:

- Though vomiting is *absent in birds*, the *retching response* involves:
  - *Central activation* of brainstem emetic nuclei (AP, NTS).
  - Involvement of *5-HT<sub>3</sub>*, *NK<sub>1</sub>*, and *dopaminergic pathways*.
- Responses are suppressed by classical *antiemetic agents*, making it useful for *neurobehavioral screening* [52].

#### Limitations:

- Lack of actual vomiting reflex limits *full translational value*.
- Requires *behavioral expertise* to distinguish between retching and other movements.
- Responses can vary with *age*, *strain*, and *handling stress*.

### 3.1.7: Emetic Reflex Model in Musk Shrew (*Suncus murinus*) (A Small Mammal Model That Possesses a Vomiting Reflex—Ideal for Low-Dose Antiemetic Screening)

#### Species Used:

- *Suncus murinus* (Musk shrew), a small insectivorous mammal.
- One of the *smallest known species* that exhibits a *true vomiting reflex*, making it highly suitable for emetic studies [55].

#### Protocol:

1. *Animal Handling & Acclimatization:*
  - Adult males/females, ~25–40 g, housed individually.
  - 12:12 light–dark cycle; temperature:  $22 \pm 2$  °C.
  - Acclimatize for at least 48 h with food and water ad libitum.
2. *Emetogen Administration:*
  - Cisplatin (10–30 mg/kg, *i.p.* or *s.c.*) – induces both *acute* (0–8 h) and *delayed* (24–72 h) emesis.
  - Other agents:
    - Copper sulfate (60–100 mg/kg, oral)
    - Nicotine, dopamine agonists, motion stimulation
3. *Observation Parameters:*
  - Observation period: 4–8 hours post-treatment.
  - Measure *number of vomits, retching frequency, latency to first episode, duration*.
  - Telemetry systems (implantable) may be used for real-time motion/emesis detection [56].
4. *Validation with Antiemetics:*
  - **Pretreatment with:**
    - Ondansetron (0.5–2 mg/kg, *i.p.*)
    - Aprepitant (NK<sub>1</sub> antagonist)
    - Metoclopramide, granisetron, dexamethasone

#### Mechanism:

- Vomiting involves coordinated activation of the *abdominal muscles, diaphragm, and oropharyngeal structures*.
- Stimulus is relayed from *vagal afferents* (via 5-HT<sub>3</sub> or NK<sub>1</sub> receptor activation) to *area postrema (AP)* and *nucleus tractus solitarius (NTS)*—similar to *ferret and human pathways* [57].

#### Limitations:

- Requires *special handling and training*, as shrews are delicate.
- High metabolic rate → *more sensitive to dosing errors*.
- Smaller blood volumes limit *pharmacokinetic sampling*.

### 3.1.8: Pigeon and Cockroach-Based Reflex Models for Emesis Research (Exploratory and Mechanistic Insights Using Non-Mammalian Vertebrate and Invertebrate Models)

#### Species Used:

- Pigeon (*Columba livia*) – avian model with brainstem emetic nuclei.
- Cockroach (*Periplaneta americana*) – invertebrate model used for *neuropharmacological* emesis-like reflex studies. Though less commonly used, these models offer valuable insights into *primitive or core emetic circuits* and *receptor-level interactions*.

#### A. Pigeon-Based Emesis Model

#### Protocol:

1. *Animal Preparation:*
  - Adult *Columba livia*, 300–400 g.
  - Acclimatize for 5–7 days in controlled aviary environment.
2. *Induction of Emesis-Like Behavior:*
  - Ipecac syrup (0.5–1.5 mL/kg, oral) or apomorphine (0.5–1 mg/kg, *s.c.*).
  - Drugs administered after brief fasting (4–6 h).
  - Monitor for signs of *regurgitation, retching, gaping, and head movements*.

3. *Endpoints Recorded:*
  - Latency to first retch/regurgitation
  - Number of retches in 60–120 min
  - Associated behaviors (gaping, throat contractions, vomiting-like expulsion)
  - Neural activation markers (c-Fos in area postrema)
4. *Antiemetic Validation:*
  - Pretreatment with *metoclopramide*, *ondansetron*, *haloperidol* reduces emesis markers.

#### Mechanism:

- Functional *area postrema* and *NTS* in pigeons allow partial modeling of central emetic reflexes [59].
- $D_2$ ,  $5-HT_3$ , and  $H_1$  receptor pathways are involved.
- Regressed vomiting reflex still allows assessment of *neural emetic circuitry*.

### B. Cockroach-Based Reflex Screening (Invertebrate Model)

#### Protocol:

1. *Species:* *Periplaneta americana* or *Blatta orientalis*
2. *Procedure:*
  - Topical or injection exposure to neuroactive agents (e.g., nicotine, histamine, serotonin).
  - Use electrophysiological setups to monitor changes in *esophageal motility*, *salivary secretions*, and *pharyngeal contractions*.
  - Observation of *regurgitation reflex*, which mimics vomiting endpoints in higher taxa [60].
3. *Advantages:*
  - Cost-effective and reproducible
  - Suitable for preliminary high-throughput neuropharmacological screening
  - Investigates *peripheral emetogen-induced motor reflexes*

#### Usefulness:

Pigeon      Emesis-like neural mapping; avian comparative anatomy insights

Cockroach: Reflex-based model for invertebrate neural pharmacology

#### Limitations:

- *Pigeons lack a complete vomiting reflex*, limiting translational predictiveness.
- *Cockroach model* does not represent mammalian neurogastroenterology.
- Mostly suited for *comparative mechanistic studies*, not for drug development validation.

### 3.1.9: Zebrafish Larvae Model for Nausea-like Behavior and Antiemetic Screening (An Emerging Vertebrate System for High-Throughput, Mechanism-Based Antiemetic Studies)

#### Species Used:

- *Danio rerio* (Zebrafish), particularly *larval stage* (4–7 days post-fertilization)
- Though zebrafish *cannot vomit*, their *nausea-like responses* and well-characterized *neurobehavioral readouts* offer a *robust surrogate system*.

#### Protocol:

1. *Larval Handling:*
  - Zebrafish larvae (96–120 hours post-fertilization) maintained in E3 medium (28 °C, 14:10 h light/dark cycle).
  - Acclimatize in 24-well or 96-well plates.
2. *Induction of Nausea-like Behavior:*
  - Exposure to *emetogens* such as:
    - *Copper sulfate* (10–50  $\mu$ M)
    - *Cisplatin* (10–100  $\mu$ M)
    - *Mustard oil* (allyl isothiocyanate)
    - *Quinine*
  - Administered via immersion for 30–60 minutes.
3. *Behavioral Readouts (Quantifiable Endpoints):*
  - *Hyperactivity or hypolocomotion*
  - *Erratic movement, darting behavior*

- Freezing, yawning-like gill movements
- Recorded using *video tracking software* or automated behavior analyzers [62].
- 4. *Antiemetic Validation:*
  - **Pre-treatment with:**
    - Ondansetron (0.5–5  $\mu$ M)
    - Aprepitant
    - Haloperidol
    - Scopolamine
  - Measures reversal of behavior anomalies [63].

#### Mechanism:

- Zebrafish possess *orthologs of human emetogenic receptors*:
  - 5-HT<sub>3</sub>, D<sub>2</sub>, NK<sub>1</sub>, H<sub>1</sub>, muscarinic receptors [64].
- CNS regions analogous to *area postrema*, *hypothalamus*, and *hindbrain nuclei* regulate *nausea-like responses*.
- Behavioral endpoints are mediated by *serotonergic and dopaminergic* circuits—confirmed by gene expression and imaging studies.

#### Limitations:

- Zebrafish *cannot vomit*, so it's not suitable for direct emetic quantification.
- Behavior-based readouts may be influenced by *non-specific neurotoxicity*.
- Validation still evolving for *clinical translatability* in humans.

### 3.2 In Vivo Models in Rats (Non-vomiting species)

#### 3.2.1 Kaolin-Induced Pica in Rats and Mice

##### 1. Species Used:

- Wistar Rats
- Sprague-Dawley Rats
- Swiss Albino Mice

##### 2. Experimental Protocol:

Kaolin (aluminum silicate clay) is mixed with water, molded into pellets, dried, and presented in a separate container next to standard food pellets.

- *Baseline Measurement:* Record kaolin and food consumption for 48 hours before test.
- *Test Treatment:* Administer an emetogenic agent like *cisplatin* (6 mg/kg i.p.).
- *Observation Period:* Measure kaolin intake at 24, 48, and 72 hours post-treatment.
- *Antiemetic Validation:* Groups pre-treated with agents like *ondansetron* (1 mg/kg i.p.), *aprepitant*, or *scopolamine* are assessed for reduction in kaolin consumption.

##### 3. Mechanism of Emesis:

Although rodents *lack the emetic reflex*, kaolin consumption mimics *nausea-like behavior*. The mechanism involves:

- Activation of *enterochromaffin cells* in the gut
- *Serotonin (5-HT)* release, acting on 5-HT<sub>3</sub> receptors of vagal afferents
- Signal transmission to the *nucleus tractus solitarius (NTS)* and *area postrema*  
This response is considered analogous to the *visceral malaise* experienced in chemotherapy-induced nausea in humans [66], [67].

##### 4. Validation and Application:

- *Validated by multiple studies* showing increased kaolin intake following cisplatin or LiCl administration.
- *Ondansetron*, *dexamethasone*, *NK<sub>1</sub> receptor antagonists*, and *ginger extracts* have all shown *dose-dependent reduction* in pica behavior [68], [69].
- Reflects both *acute (within 24 h)* and *delayed (>24 h)* phases of nausea.
- A *non-invasive, cost-effective, and ethically favorable* model for high-throughput antiemetic drug screening in non-vomiting species.

### 3.2.2 Conditioned Taste Aversion (CTA) in Rats

#### 1. Species Used:

- *Wistar Rats*
- *Sprague-Dawley Rats*

#### 2. Experimental Protocol:

Rats are presented with a *novel-tasting solution* (commonly 0.1% saccharin or sodium chloride) for a short duration (usually 15–30 minutes).

- *Conditioning Phase:* After drinking the novel solution, rats receive an intraperitoneal injection of an *emetogen* like *lithium chloride (LiCl, 0.15 M, 20 ml/kg i.p.)* or *cisplatin (6 mg/kg i.p.)*.
- *Test Phase (24–48 h later):* Rats are given a *choice between the novel solution and water*.
- *Outcome:* A reduction in intake of the novel taste indicates *conditioned aversion*, interpreted as a *behavioral correlate of nausea* [70].

#### 3. Mechanism of Emesis:

CTA does not measure vomiting directly but *reflects anticipatory or learned nausea*. It engages:

- *Limbic system structures* such as the *amygdala, insula, and gustatory cortex*
- *Neurochemical involvement:* Includes serotonin, dopamine, and NMDA signaling in the aversion circuitry [71], [72].

#### 4. Validation and Application:

- Widely used to *study nausea in non-vomiting species*, especially where classical vomiting models are not feasible.
- Sensitive to known antiemetics like *ondansetron, aprepitant, corticosteroids, and cannabinoids* [73].
- Also used to investigate *anticipatory nausea*, which is difficult to evaluate in other models.
- Important in exploring *central mechanisms* of nausea and *affective components* of the emetic reflex.

### 3.2.3 Gaping Response in Rats

#### 1. Species Used:

- *Sprague-Dawley Rats*
- *Wistar Rats*

#### 2. Experimental Protocol:

The *gaping response* is measured using *intraoral infusion* or *taste reactivity tests*:

- Rats receive *intraoral delivery* of a conditioned taste stimulus (e.g., *saccharin* or *sucrose*) that had previously been paired with an *emetogen* (e.g., *LiCl, 0.15 M, 20 ml/kg i.p.*).
- The rat's *orofacial reactions* are recorded using *high-speed video*, focusing on *gaping (wide, triangular mouth opening)*.
- *Gaping is scored* over a time window (e.g., 5–10 min).
- Administering antiemetics (e.g., *ondansetron, haloperidol, or cannabinoids*) prior to emetogen challenge *reduces gaping frequency*, validating the model [74].

#### 3. Mechanism of Emesis:

Although rats cannot vomit, the gaping response reflects *visceral malaise and nausea*:

- Involves activation of *brainstem nuclei*, including *nucleus tractus solitarius (NTS)*
- Modulated by the *insular cortex, amygdala, and ventral tegmental area (VTA)*
- Neurochemical pathways: *5-HT<sub>3</sub>, D<sub>2</sub>, NK<sub>1</sub>, CB<sub>1</sub>, and opioid receptors* [75], [76]

#### 4. Validation and Application:

- Validated for *central and peripheral emetogens*: LiCl, cisplatin, nicotine, and radiation exposure.
- Sensitive to *classic and novel antiemetics*, including *serotonin antagonists*, *cannabinoids*, and *NK<sub>1</sub> blockers*.
- Useful in *anticipatory and conditioned nausea* studies.
- Frequently applied to screen *non-emetic nausea modulators*, including herbal and endogenous neuromodulators [77].

#### 3.2.4 Facial Grooming and Licking Behavior in Rodents

##### 1. Species Used:

- Mice (e.g., C57BL/6, Swiss albino)
- Rats (Wistar, Sprague-Dawley)

##### 2. Experimental Protocol:

This model uses *behavioral scoring* of grooming and licking patterns as indirect indicators of *nausea-like states*, particularly after administration of emetogens such as *cisplatin* (6–10 mg/kg i.p.), *LiCl*, or *opioids*:

- *Behavioral Monitoring*: After drug administration, animals are placed in transparent enclosures and recorded for 30–60 minutes.
- *Endpoints Observed*:
  - Excessive *facial grooming*
  - *Forepaw licking*
  - *Head shaking*
  - *Immobilization or hunched posture*
- Anti-emetic agents (e.g., *ondansetron*, *cannabinoids*) are used to confirm model sensitivity by reducing the intensity or frequency of these behaviors [78], [79].

##### 3. Mechanism of Emesis:

Though rodents lack a vomiting reflex, they still *experience nausea-like distress*, indicated by:

- Activation of *serotonergic* (5-HT<sub>3</sub>) and *dopaminergic* (D<sub>2</sub>) pathways
- Central modulation by *insular cortex*, *area postrema*, and *parabrachial nucleus*
- Autonomic symptoms (e.g., piloerection, reduced locomotion) co-occur with behavioral signs [80]

#### 4. Validation and Application:

- Used in *early-phase antiemetic screening*, especially when classical vomiting isn't measurable.
- Particularly useful for assessing *peripheral nausea*, chemotherapy-induced malaise, or vestibular disturbance.
- Ideal for *chronic dosing*, behavioral phenotyping, and *genetically modified mice*.
- Considered more *naturalistic and non-invasive* compared to CTA or pica models [81].

#### 3.2.5 Open Field and Immobility Behavior in Mice (Motion/Nausea-Linked Anxiety Model)

##### 1. Species Used:

- Swiss Albino Mice
- C57BL/6 Mice
- BALB/c Mice

##### 2. Experimental Protocol:

This behavioral model evaluates *locomotor suppression* and *freezing/immobility* in response to motion-related stimuli or nausea-inducing agents:

- *Setup*: Mice are placed in an *open-field arena* (e.g., 40 × 40 cm) after administration of emetogens (e.g., *cisplatin* 10 mg/kg i.p., *LiCl* 0.15 M, or *motion exposure* via shaking or tilting platforms).
- *Observation Duration*: 5–10 minutes post-treatment.

- *Parameters Measured:*
  - *Total distance moved* (via video tracking systems)
  - *Number of rearings*
  - *Duration of immobility (freezing)*
  - *Center vs. periphery time* as a measure of anxiety
- Anti-nausea drugs like *ondansetron*, *scopolamine*, or *CB1 agonists* reduce immobility and increase exploratory behavior, indicating *behavioral recovery* [82], [83].

### 3. Mechanism of Emesis:

Though mice cannot vomit, *nausea-like anxiety* and behavioral suppression occur due to:

- Activation of *vestibular and limbic circuits* (especially *amygdala, hippocampus*)
- Involvement of *serotonin, histamine, and acetylcholine* pathways
- Increased *hypothalamic-pituitary-adrenal (HPA)* axis activity during nausea [84]

### 4. Validation and Application:

- Acts as a *behavioral proxy* for motion-induced nausea.
- Sensitive to *motion sickness medications* (H1/M1 antagonists like *promethazine, scopolamine*).
- Useful in studies involving *space motion, virtual reality motion sickness*, or *anti-nausea herbal extracts*.
- Valuable for *non-invasive and real-time screening*, especially in *genetically altered mice* [85].

#### 3.3. Ex Vivo / In Vitro Models for Antiemetic Screening

These models are critical in the *early stages of drug development*, allowing researchers to *study molecular mechanisms, receptor interactions*, and *functional responses* to emetogenic stimuli in a *controlled environment* without involving live animals. While they *cannot replicate full-body emetic responses*, they provide *mechanistic insights* and guide *compound optimization*.

##### 3.3.1 Receptor Binding Assays

#### 1. Species Used:

- Human recombinant receptors (cloned into CHO or HEK-293 cells)
- Rat or guinea pig brain membrane preparations

#### 2. Experimental Protocol:

- Ligands (test compounds) are incubated with *radiolabeled standard ligands* (e.g., [<sup>3</sup>H]-GR65630 for 5-HT<sub>3</sub>, [<sup>3</sup>H]-spiperone for D<sub>2</sub>) in a receptor-rich membrane preparation.
- Binding displacement curves are plotted to calculate *IC<sub>50</sub>/K<sub>i</sub>* values.
- Employed in *high-throughput screening (HTS)* for compounds targeting 5-HT<sub>3</sub>, D<sub>2</sub>, NK<sub>1</sub>, CB<sub>1</sub>, etc. receptors [86], [87].

#### 3. Mechanism Assessed:

- Measures *affinity and specificity* of test compounds to emetogenic targets.
- Predicts *potential antiemetic efficacy* before in vivo validation.
- Allows for *structure–activity relationship (SAR)* studies.

#### 4. Validation and Application:

- All clinically used antiemetics (e.g., *ondansetron, metoclopramide, aprepitant*) were optimized via these assays.
- Assays have excellent *predictive value for central and peripheral targets*.
- Used widely in *lead optimization, receptor selectivity profiling, and off-target screening* [88].

### 3.3.2 Isolated Tissue Contractility Models (e.g., Ileum, Fundus)

#### 1. Species Used:

- Guinea pig ileum
- Rat stomach fundus
- Rabbit jejunum

#### 2. Experimental Protocol:

- Tissue strips are mounted in *organ baths* filled with physiological solution at 37°C and gassed with *carbogen* (95% O<sub>2</sub> + 5% CO<sub>2</sub>).
- Baseline tension is recorded.
- Emetogens (e.g., 5-HT, dopamine, substance P) are applied cumulatively to generate *dose–response curves*.
- *Test compounds* are added to observe their *inhibitory effects on contractile response* [89], [90].

#### 3. Mechanism Assessed:

- *Functional receptor responses* (5-HT<sub>3</sub>, D<sub>2</sub>, NK<sub>1</sub>) based on *smooth muscle contraction*.
- *Mimics peripheral emetogenic stimulation*.

#### 4. Validation and Application:

- Used to validate antiemetics like *granisetron*, *metoclopramide*, and *aprepitant*.
- Particularly useful in *screening natural compounds* or herbal extracts for antiemetic potential.
- Can assess *agonist–antagonist interaction* at specific receptors [91].

### 3.3.3 Brainstem Slice Studies (Area Postrema & Nucleus Tractus Solitarius)

These models offer a direct way to study *central emetic circuitry*, particularly in regions known as the "*vomiting centers*", such as the *Area Postrema (AP)* and *Nucleus Tractus Solitarius (NTS)*. This is particularly useful for testing *central-acting antiemetics* and decoding neurochemical signaling.

#### Brainstem Slice Electrophysiology & Neurochemical Mapping

#### 1. Species Used:

- Neonatal or juvenile *Sprague–Dawley rats*
- Adult *mice* (*wild-type* or *knockout*)
- In some studies, *guinea pigs*

#### 2. Experimental Protocol:

- After euthanasia, *coronal or horizontal brainstem slices* (250–400 μm) are prepared using a *vibratome* in cold oxygenated artificial cerebrospinal fluid (aCSF).
- The slices contain *AP*, *NTS*, or *dorsal vagal complex (DVC)*.
- *Whole-cell patch-clamp electrophysiology* or *calcium imaging* is used to monitor neuronal responses.
- *Application of emetogens* (e.g., 5-HT, substance P, dopamine) or antiemetics (e.g., ondansetron, aprepitant) is done via bath perfusion.
- Sometimes coupled with *immunohistochemistry* for c-Fos or neurotransmitter markers [92], [93].

#### 3. Mechanism Assessed:

- *Direct recording of neuronal excitability* and synaptic transmission within key emetic centers.
- Differentiation of *neuron subtypes* (glutamatergic, GABAergic, serotonergic).
- Mechanisms such as *NK<sub>1</sub> receptor desensitization*, *5-HT<sub>3</sub> ion channel blockade*, and *CB<sub>1</sub>-mediated hyperpolarization* can be evaluated [94].

#### 4. Validation and Application:

- Validated by multiple studies examining *palonosetron*, *metoclopramide*, and *capsaicin*'s desensitization effect.
- Used to dissect *developmental changes*, *drug tolerance*, and *cross-talk between emetic neurotransmitters*.
- Allows *precise localization of drug effects*, important in *drug design targeting central emetic reflex*.
- Useful in confirming results from *in vivo emetic models* [95].

#### 3.3.4 Human-Derived Organoid and iPSC-Based Models for Antiemetic Research

This cutting-edge model type leverages human stem cell technologies to replicate gastrointestinal and neural tissues involved in emesis. These systems serve as *translationally relevant, species-independent tools* for antiemetic drug screening and mechanistic studies.

Human GI Organoids and Brain-Gut Axis Models

##### 1. Species/Source Used:

- *Human induced pluripotent stem cells (hiPSCs)*
- *Human embryonic stem cells (hESCs)*
- *GI tissues/organoids* derived from patients or donors

##### 2. Experimental Protocol:

- *Differentiation of hiPSCs* into:
  - Enteroendocrine-like cells (EECs) containing *enterochromaffin (EC) cells*
  - Neural progenitor cells that simulate *vagal afferent pathways*
- *3D intestinal organoids* are matured in Matrigel domes or microfluidic chips for weeks.
- Organoids are *exposed to emetogens* such as *cisplatin*, *5-HT*, or *substance P*, and antiemetics (e.g., *ondansetron*, *netupitant*) are added to assess blockade.
- Endpoints include:
  - *Serotonin (5-HT) secretion* (via ELISA or amperometry)
  - *Gene expression changes* (via qPCR/RNA-seq)
  - *Calcium flux or voltage recordings* in neuro-organoids [96], [97].

##### 3. Mechanism Assessed:

- Primarily simulates the *enterochromaffin–vagal afferent axis*.
- Allows study of *5-HT<sub>3</sub>*, *NK<sub>1</sub>*, *TRPV1*, and *opioid receptor involvement*.
- Interrogates *drug–receptor–neuron interactions* in a human background.
- Facilitates *epigenetic, transcriptomic, and proteomic* analysis in emesis models [98].

#### 4. Validation and Application:

- Demonstrated *5-HT* release in response to *cisplatin* or radiation exposure [99].
- *Ondansetron* and *granisetron* shown to suppress *5-HT* surge in EEC-organoids.
- *NK<sub>1</sub>* antagonist *aprepitant* reduces *substance P*-mediated responses in neural co-culture [100].
- Used to *screen plant-derived antiemetics* and assess human-relevant toxicity [101].
- Emerging use in *precision antiemetic screening* (e.g., patient-derived organoids) [102].

#### 3.4 Human Provocation Models for Antiemetic Evaluation

Human provocation models are ethically controlled experimental paradigms used to evaluate nausea and emesis in volunteers. These models offer *high translational relevance, non-invasive assessment*, and real-time subjective and physiological measurements, making them pivotal for Phase I/II trials.

##### 3.4.1 Ipecac-Induced Nausea and Emesis in Healthy Volunteers

###### 1. Species Used:

- Healthy human volunteers (adult males/females, 18–45 years)

- Strict inclusion/exclusion criteria (non-pregnant, non-smokers, no GI/psychiatric illness)

## 2. Experimental Protocol:

- *Ipecac syrup* (10–15 mL diluted in 100 mL water) is orally administered.
- Onset of nausea typically begins within 20–30 minutes, followed by vomiting in most participants.
- *Visual Analog Scale (VAS)* and numeric nausea scores (0–100 scale) are recorded every 5–10 min.
- Emesis parameters (latency, number of episodes, duration) are tracked.
- Rescue with antiemetic (ondansetron 4–8 mg) after protocol ends [103],[104].

## 3. Mechanism of Emesis:

- Ipecac stimulates gastric mucosa and activates vagal afferents → NTS (nucleus tractus solitarius) → area postrema activation → emetic reflex.
- Also stimulates serotonin release, mimicking 5-HT<sub>3</sub>-dependent nausea pathways [105].

## 4. Validation and Application:

- Commonly used in early-phase antiemetic testing (e.g., 5-HT<sub>3</sub> antagonists, cannabinoids, herbal agents).
- Ondansetron, metoclopramide, and ginger extracts have been validated using this model [106].
- Used to compare onset and efficacy between antiemetic agents in crossover studies.
- Standard for controlled nausea induction with ethical committee approvals and safety protocols [107].

### 3.4.2 Visual Analog Scale (VAS) for Nausea Monitoring

#### 1. Species Used:

- Human participants across various antiemetic and motion sickness studies.
- Applicable in clinical trials, volunteer challenge studies, and cancer therapy monitoring.

#### 2. Experimental Protocol:

- Participants are provided a 100 mm horizontal line, anchored with “No Nausea” (0) on the left and “Worst Possible Nausea” (100) on the right.
- They are instructed to mark their perceived level of nausea at regular intervals during exposure to an emetic stimulus (e.g., motion, ipecac, visual rotation).
- Scores are recorded every 5–10 minutes or at predefined endpoints (e.g., 30, 60, 90 minutes).
- VAS can be used standalone or in conjunction with physiological markers like heart rate, skin conductance, and EEG [108].

#### 3. Mechanism of Emesis:

- The VAS does not provoke nausea, but rather quantifies the subjective sensation of nausea.
- It reflects cortical and limbic processing of emetic stimuli.
- Often used in parallel with provocation models (e.g., motion, ipecac) [109].

#### 4. Validation and Application:

- Used to assess real-time antiemetic efficacy of drugs like ondansetron, ginger, promethazine, and scopolamine.
- Validated across studies involving vestibular stimulation, chemotherapy-induced nausea, and anticipatory nausea [110].
- Allows non-invasive, quantifiable, and repeatable assessment of nausea intensity.
- Forms a standard clinical endpoint in antiemetic drug trials [111].

### 3.4.3 fMRI-Based Nausea Detection Models

#### 1. Species Used:

- Human subjects (healthy volunteers or patients undergoing chemotherapy, motion simulation, or drug studies).

- Non-invasive neuroimaging tool used to explore *central nervous system correlates of nausea*.

## 2. Experimental Protocol:

- Participants are exposed to nausea-inducing stimuli such as:
  - *Rotating visual scenes*,
  - *Vestibular conflict via motion platforms*,
  - *Chemically induced nausea* (e.g., ipecac, apomorphine)
- Functional MRI is conducted during or immediately after exposure.
- BOLD (Blood-Oxygen-Level Dependent) signals are recorded and analyzed, particularly in the *insula*, *anterior cingulate cortex*, *parabrachial nucleus*, and *amygdala* [112], [113].

## 3. Mechanism of Emesis:

- Identifies *activation patterns in brain regions* involved in nausea perception and autonomic control.
- The *insula* is central for integrating *visceral signals*, and the *amygdala* is linked to the *emotional processing of nausea*.
- *Parabrachial nucleus and brainstem areas* contribute to autonomic outputs like salivation, gastric dysrhythmias, and retching [114].

## 4. Validation and Application:

- Validated in studies comparing BOLD signals during exposure to motion, visual-vestibular conflict, or pharmacologic emesis [115].
- Enables *mechanism-based profiling* of antiemetics by monitoring *brain deactivation patterns* during treatment [116].
- Used in evaluating new central-acting antiemetics, *neurofeedback therapies*, and *neuromodulation approaches* (e.g., transcranial stimulation) [117].

### 3.4.4 Virtual Reality (VR)-Induced Nausea Models

#### 1. Species Used:

- *Human subjects*, primarily healthy adult volunteers or individuals prone to *motion sickness*, used in controlled lab or simulated settings.
- Also applied in *chemotherapy patients* to investigate anticipatory nausea and in *aerospace/military medicine* contexts.

#### 2. Experimental Protocol:

- Participants are immersed in a *head-mounted display (HMD)* system or *VR dome projection*, presenting rotating/oscillating environments, often inducing *visuo-vestibular mismatch*.
- Sessions last *5–30 minutes*, with the *Simulator Sickness Questionnaire (SSQ)* and *Visual Analog Scale (VAS)* used for scoring nausea intensity [118].
- Additional variables like *head-tracking lag*, *field of view*, and *movement latency* are manipulated to modulate nausea .

#### 3. Mechanism of Emesis:

- Induces *cyber sickness*, a subtype of motion sickness, caused by *conflicting sensory inputs* between visual and vestibular systems.
- Activation of *vestibular nuclei*, *cerebellum*, and *brainstem autonomic centers* leads to nausea, cold sweats, pallor, and retching .
- Closely mimics motion-induced emesis and is useful for *predicting susceptibility to kinetosis or anticipatory nausea*.

#### 4. Validation and Application:

- Validated using *antiemetic interventions* like *scopolamine*, *ginger*, and *behavioral acclimatization protocols* .
- Used to study *dose-response* relationships of antiemetics in non-invasive human studies.
- Extensively applied in the *gaming industry*, *aerospace medicine*, and *rehabilitation* research.
- Provides real-time measurement and is modifiable for individualized sensitivity .

### 3.4.5 Gastrointestinal Barostat-Based Nausea Models

#### 1. Species Used:

- *Humans (healthy volunteers or patients)* undergoing intragastric balloon distension procedures.
- Some preliminary work also in *animal models* (e.g., pigs, dogs) using implanted barostat balloons.

#### 2. Experimental Protocol:

- A *barostat device* controls the inflation of an intragastric balloon at variable pressures (e.g., 2–20 mmHg) to *mimic gastric distension*.
- Nausea, fullness, and discomfort are recorded using *Visual Analog Scales (VAS)* or *nausea questionnaires* during the stepwise inflation process.
- Balloon pressure/volume curves are recorded to assess *visceral sensitivity thresholds*.
- Duration: Each session typically lasts 45–90 minutes, with incremental pressure increases every 5–10 minutes.

#### 3. Mechanism of Emesis:

- Gastric distension stimulates *mechanosensitive afferents* of the *vagus nerve*, relaying signals to the *nucleus tractus solitarius (NTS)* and *area postrema* in the brainstem.
- Closely mirrors *gastric dysrhythmias and bloating-induced nausea*, especially in disorders like *functional dyspepsia*, *gastroparesis*, and *post-chemotherapy nausea*.
- Does *not cause vomiting* directly but is a robust, quantifiable model of *gastric discomfort and nausea*.

#### 4. Validation and Application:

- Validated by comparing responses to *ondansetron*, *metoclopramide*, and *acupuncture therapy*.
- Used in evaluating *neuromodulators*, *visceral analgesics*, and *prokinetic agents*.
- Helps in characterizing *visceral hypersensitivity* and in studying *gut–brain axis* dysfunctions.
- Particularly useful in *functional GI disorders*, including *IBS* and *functional nausea syndromes*.

## 4. Comparative Analysis and Model Selection Matrix

This section compares antiemetic models across species and protocols in terms of *mechanistic relevance*, *translatability*, *ethical viability*, and *application stage* in drug development. The goal is to guide researchers in choosing the *most appropriate model* based on their *study objective* be it mechanistic discovery, screening, or translational application.

### 4.1 Comparative Table of In Vivo Emesis Models

| Model                         | Species               | Mechanism Targeted                                                    | Translational Validity | Emetic/Nausea Endpoint | Ethical Feasibility | Best Suited For                                                           |
|-------------------------------|-----------------------|-----------------------------------------------------------------------|------------------------|------------------------|---------------------|---------------------------------------------------------------------------|
| Cisplatin-Induced Emesis      | Ferret, Dog, Monkey   | 5-HT <sub>3</sub> /NK <sub>1</sub> pathways (CINV)                    | High                   | Vomiting               | Moderate            | Chemotherapy-induced emesis studies [21–25]                               |
| Apomorphine-Induced Emesis    | Dog, Ferret           | D <sub>2</sub> receptor (central)                                     | Moderate               | Vomiting               | Good                | Dopaminergic-target drug screening [26–28]                                |
| Radiation-Induced Emesis      | Ferret, Monkey        | Delayed emesis (CINV, RINV)                                           | High                   | Vomiting               | Low                 | Radiation therapy models [29–30]                                          |
| Copper Sulfate-Induced Emesis | Cat, Dog              | Peripheral GI irritation                                              | Moderate               | Vomiting               | Good                | GI-toxicant screening [71–73]                                             |
| Motion-Induced Emesis         | Dog, Cat, Monkey      | Vestibular–cerebellar circuits                                        | High                   | Vomiting               | Good                | Motion sickness therapy (H <sub>1</sub> /M <sub>1</sub> blockers) [38–40] |
| Chick Retching Model          | Chick (Gallus gallus) | NK <sub>1</sub> /5-HT <sub>3</sub> /D <sub>2</sub> modulation         | Moderate               | Retching frequency     | Excellent           | Neurokinin-1 studies, low-cost emetic model [76–79]                       |
| Musk Shrew Model              | <i>Suncus murinus</i> | 5-HT <sub>3</sub> /NK <sub>1</sub> /D <sub>2</sub> -mediated vomiting | High                   | Vomiting               | Good                | Small rodent with true emesis; CINV mimic [81–83]                         |

|                               |                  |                                                         |          |                           |           |                                         |
|-------------------------------|------------------|---------------------------------------------------------|----------|---------------------------|-----------|-----------------------------------------|
| Zebrafish Larval Nausea Model | Zebrafish larvae | 5-HT <sub>3</sub> /NK <sub>1</sub> -mediated locomotion | Moderate | Swimming patterns (proxy) | Excellent | High-throughput early screening [63–65] |
|-------------------------------|------------------|---------------------------------------------------------|----------|---------------------------|-----------|-----------------------------------------|

4.2 Surrogate and Non-Emetic Models Comparison

| Model                             | Species   | Nausea Endpoint                    | Proxy | Mechanistic Focus                       | Translational Use | Strengths                                    | References |
|-----------------------------------|-----------|------------------------------------|-------|-----------------------------------------|-------------------|----------------------------------------------|------------|
| Kaolin-Induced Pica               | Rats/Mice | Kaolin intake (grams/day)          |       | Visceral nausea/serotonergic            | High              | Quantifiable; easily replicable              | [31–34]    |
| Conditioned Taste Aversion (CTA)  | Rats/Mice | Taste avoidance                    |       | Anticipatory/emotional nausea           | Moderate          | Good limbic relevance                        | [35–37]    |
| Gaping Behavior                   | Rats      | Orofacial movements                |       | Limbic/anticipatory nausea              | Moderate          | Excellent nausea mimic in non-emetic species | [36–37]    |
| Gastric Barostat Distension Model | Humans    | Discomfort, VAS, GI pressure       |       | Visceral hypersensitivity               | High              | Best for dyspeptic nausea, gut-brain studies | [123–127]  |
| Ipecac-Induced Emesis             | Humans    | Nausea & emesis via VAS scoring    |       | 5-HT <sub>3</sub> , sensory stimulation | High              | Real-time validation in volunteers           | [103–106]  |
| Virtual Reality (VR) Nausea Model | Humans    | VAS, heart rate, facial expression |       | Visual–vestibular mismatch              | Moderate          | Motion sickness and anticipatory nausea      | [112–114]  |
| fMRI-Based Nausea Mapping         | Humans    | BOLD signal in insula/amygdala     |       | Limbic and cortical circuits            | High              | CNS correlates of nausea in real time        | [109–111]  |

4.3 Model–Target–Drug Matching Matrix

| Model Type                | Key Target                          | Best Drug Classes Tested                 | Example Drug(s)           | Phase of Research            |
|---------------------------|-------------------------------------|------------------------------------------|---------------------------|------------------------------|
| Ferret (Cisplatin Model)  | 5-HT <sub>3</sub> , NK <sub>1</sub> | Setrons, Aprepitant, Corticosteroids     | Ondansetron, Aprepitant   | Preclinical to Phase II [21] |
| Motion Sickness (Dog/Cat) | H <sub>1</sub> , M <sub>1</sub>     | Antihistamines, Anticholinergics         | Promethazine, Scopolamine | Translational [38]           |
| Apomorphine (Dog/Ferret)  | D <sub>2</sub>                      | Dopaminergic antagonists                 | Metoclopramide            | Mechanistic screening [27]   |
| Chick Retching            | NK <sub>1</sub> , 5-HT <sub>3</sub> | Neurokinin blockers, Setrons             | CP-99,994                 | Early screening [76]         |
| Kaolin Pica (Rat)         | 5-HT <sub>3</sub> , Central         | Setrons, NK <sub>1</sub> antagonists     | Ondansetron, Aprepitant   | Surrogate testing [31]       |
| Zebrafish                 | Serotonergic/GABAergic              | Novel ligands, herbal compounds          | GABA analogues            | High-throughput early [63]   |
| VR-induced Nausea (Human) | Vestibular, Limbic                  | M <sub>1</sub> antagonists, cannabinoids | Scopolamine, Nabilone     | Phase I–II [112]             |

5 Conclusion

The preclinical evaluation of antiemetic therapies relies on a diverse array of experimental models that reflect the intricate neurobiological mechanisms underlying nausea and vomiting. This review consolidates and categorizes these models—ranging from emetic species like ferrets, dogs, cats, monkeys, and shrews to surrogate non-emetic species such as rats, mice, and zebrafish larvae—highlighting their individual protocols, mechanistic relevance, and translational value. Each model captures distinct emetogenic pathways including serotonergic, dopaminergic, neurokinin, vestibular, and cortical inputs, which are crucial for evaluating class-specific antiemetic drugs such as 5-HT<sub>3</sub> antagonists, NK<sub>1</sub> receptor blockers, dopamine antagonists, antihistamines, and corticosteroids. Behavioral surrogates like kaolin pica, conditioned taste aversion, and gaping responses serve as indispensable alternatives in species lacking a vomiting reflex, providing ethically feasible and mechanistically informative tools. The review also emphasizes the need for *model selection based on mechanistic alignment* with the clinical condition of interest—such as chemotherapy-induced nausea and vomiting (CINV), radiation sickness, motion sickness, or anticipatory nausea—thereby enhancing predictive validity. Moreover, emerging tools like *telemetry systems*, *functional imaging*, *in vitro gut-brain axis platforms* and *human provocation models* offer promising directions for bridging translational gaps.

In conclusion, no single model suffices for all antiemetic evaluations. An integrated, mechanism-driven, and condition-specific model selection strategy—augmented by behavioral, molecular, and computational tools—is vital to advancing antiemetic drug discovery. Standardizing protocols, validating newer surrogate endpoints, and incorporating multi-species approaches will pave the way for more predictive and ethically sound translational research.

## 6. Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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