

Chinese Journal of Cancer Prevention and Treatment

eISSN: None / ISSN: 1673-5269 Open Access

Review Article

Beyond Viability: Why Caco-2-Based Studies of Multi-Walled Carbon Nanotubes Need Barrier, Transporter, and Molecular Endpoints

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Article History
Received: 10. 09. 2026
Revised: 22. 09. 2026
Accepted: 20. 10. 2026
Published: 05. 11. 2026

Citations:
Tiwari Y, Surendra G, Male A, Mishra A, Vinode R, Shrivastava C. Beyond viability: why Caco-2-based studies of multi-walled carbon nanotubes need barrier, transporter, and molecular endpoints. Chin. J. Cancer Prev. Treat, 33(11) 09-26, 2026.

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Abstract: Multi-walled carbon nanotubes (MWCNTs) are high-aspect-ratio carbon materials that are employed in the field of composites, electronics, environmental technologies and biomedical research. For both safety assessment and application development, intestinal models are relevant because they can be used for studying the intestinal absorption of these agents and their potential as drug carriers. The evidence of MWCNT interaction with Caco-2 cells, other studies involving carbon nanotubes in intestinal models and the experimental conditions that affect the results reported are explored in this narrative review. There is limited evidence as a direct basis. It contains studies that have reported negligible acute viability loss or uptake in Caco-2 cells during testing, proteomic and transcriptomic changes in the co-culture of Caco-2/HT29-MTX cells, and changes in expression of drug efflux transporters in Caco-2 cells. Other studies of single-walled carbon nanotubes (SWCNTs) and/or other carbon nanomaterials have demonstrated that effects may vary with dose, surface chemistry, dispersion, differentiation state and biological context. Hypothetical approaches can be made based on evidence from T-84 cells, animal intestines, lung models and microbiome studies, but cannot be considered as applicability evidence for Caco-2 responses. The review suggests that the use of viability as an end point in intestinal hazard evaluation of MWCNT is not sufficient. Barrier resistance and permeability, organization of tight junctions, presence of microvilli, transporter function, oxidative and inflammatory reaction, and cellular association should be measured in combination with each other.Barrier resistance and permeability, organization of tight junctions, presence of microvilli, transporter function, oxidative and inflammatory responses, and cellular association should be measured together. Well characterised materials, realistic and transparent dosimetry, differentiated monocultures and mucus-containing co-cultures, simulated digestion for when oral exposure is the question and common reporting standards are all important for progress. No definitive conclusion can be drawn on the safety or toxicity of MWCNTs to the intestinal epithelium, but there is supporting evidence in the current literature for targeted testing and careful interpretation.

Keywords: multi-walled carbon nanotubes; Caco-2; intestinal barrier; nanotoxicology; oral exposure; epithelial permeability

INTRODUCTION

Carbon nanotubes are the cylindrical carbon nanostructures that are made up of one or more concentric graphene-like tube. Single-walled carbon nanotubes are composed of one wall while multi-walled carbon nanotubes are made up of multiple nested walls. Mechanical, electrical, thermal and surface binding properties of both classes have been examined. These attributes enable applications in polymers, sensors, energy devices, coatings and experimental biomedical systems. The same properties also present a challenge in terms of safety assessment: different properties including length, diameter, curvature, aggregation, surface chemistry, metal catalyst residues and dispersibility all affect biological interactions (Donaldson et al., 2006; Johnston et al., 2010; Mohanta et al., 2019; Smart et al., 2006).

The intestine is a potential route of contact for nanomaterials that are intentionally ingested, found in foods or consumer products and accidentally ingested following clearance from the respiratory tract. The quantity that reaches the gut is dependent upon the route of exposure and material history. Exposure to an inhaled product in the workplace, through food or as an oral pharmaceutical cannot be substituted for each other.

Several reviews on gastrointestinal nanomaterial exposure have highlighted the importance of the luminal environment for altering the aggregation and surface properties of nanomaterials, and the combined effects of mucus, epithelial, immune, and microbiota cells on their uptake and response (Bergin &Witzmann, 2013; Pietroiusti et al., 2017; Qiao et al., 2024; Fröhlich & Fröhlich, 2016).

The Caco-2 cells are commonly used for modeling some of the functions of the human intestinal epithelium. They can be differentiated to establish polarized monolayers with tight junctions and brush border features and measurable transport properties. The model can be used to study permeability, uptake and epithelial response but the cell line is not a full intestinal tissue but is a colorectal adenocarcinoma derived cell line. The phenotype can be influenced by conditions of culture, passage number, differentiation period, seeding density and medium. A standard monoculture lacks a number of factors, such as mucus-producing cells, immune cells, bacteria, vasculature and mechanical forces (Artursson, 1990; Hidalgo et al., 1989; Natoli et al., 2012; Sambuy et al., 2005).

It is therefore a somewhat limited evidence base for "MWCNT and Caco-2". Only a few studies were conducted that directly tested the effect of MWCNTs on Caco-2 cells or Caco-2/HT29-MTX co-cultures. Other papers deal with SWCNTs, nanocarbons in general, T-84 intestinal cells, animal intestinal tissue or any other cell types except intestinal cells. These closely related sources may be useful in identifying candidate mechanisms and/or methods but should not be used together as though they were measuring the same material in the same model. This is an important part of the interpretation of the field.

To answer these four questions, this review will be conducted. Are there any direct evidences of MWCNT interaction with a Caco-2 based model? What are the features of the material or cell-models that are responsible for apparently different outcomes? What endpoints would be useful to measure for effects not just cell death? What experiment would more accurately produce results that would be useful for oral exposure assessment in the future? The overall take home message from existing studies is that the results are context specific; they point to potential cell and barrier effects, but not to a common safety threshold or to a general safety statement.

Review approach and scope of the project.

This paper is a narrative review and not a systematic review or meta-analysis. A search in PubMed and in records indexed by publishers was carried out and a reference list was checked. The search terms used were: multi-walled carbon nanotube, MWCNT, carbon nanotube, Caco-2, Caco-2/HT29-MTX, intestinal epithelial, intestinal barrier, oral exposure, and gut microbiota. The search period was up to 16 October 2026. Peer-reviewed primary literature studies that investigated MWCNTs in intestinal cells directly were prioritized, with closely related CNT intestinal studies and literature on Caco-2 methods and gastrointestinal nanotoxicology and MWCNT characterization.

Review will differentiate between direct and adjacent evidence. A direct study is performed using MWCNTs and Caco-2 cells or an intestinal co-culture of Caco-2 cells. The evidence cited next to each type of evidence is located near the corresponding type of evidence. The weight of the inferences of these categories varies. For instance, a pulmonary study can be used to make hypotheses on fiber length or oxidative stress, but not an oral epithelial dose-response. Similarly, there is no way to convert a T-84 barrier result to a Caco-2 result.

No attempt was made to conduct a quantitative meta-analysis. The studies available exhibit a variety of nanotube source and functionalization, dispersion methods, administered dose, cell maturation, exposure time and biological endpoints. If they were to pool their values it would be an assumption that they are comparable which has not been proven. In this paper, figures are conceptual schematics and are not a quantitative plotting of the experimental results. The evidence tables are intended to highlight representative studies and design considerations, and are not intended to systematically list all the evidence.

The literature search included mapping of the evidence and the identification of primary studies, but not estimation of an effect size. The review did not pre-register a protocol, duplicate title and abstract screening and did not use a formal Risk of Bias instrument. Thus, “selected evidence” involves studies that are selected because of their relevance to the stated question and not all studies that could be found that mention CNTs and intestinal biology. The bibliographic details and the DOI were verified against the indexed or the publishers records, when available – it is a narrative synthesis and readers should refer to original methods to set an exposure level or to select an assay. The weight of evidence statements is based on the extent to which each study is closely matched with the central question - same material class, Caco-2 containing model, oral-relevant exposure, and functionally informative outcomes. A study that compares just one or two of these dimensions is not for making a direct conclusion but rather is used to propose a hypothesis. This way, the wide-ranging term “CNT” is not seen as a single exposure, but makes it clear what exactly is being searched for and what is not.

The review also interprets the evidence using Evidence Hierarchy. Increased relevance to direct application to Caco-2 cells or to a co-culture with Caco-2 is inferred from findings obtained with MWCNTs applied directly to Caco-2 cells or in a co-culture with Caco-2. The results obtained from SWCNTs in the Caco-2 cells are used to gain insight into the behavior of the assays and possible pathways and are not used to replace the results of the MWCNTs. Data from the T-84 cells and other nanomaterials in intestinal models and non-intestinal systems are referred to as context. Animal findings answer the questions of plausibility for the oral route and the target organism and do not provide a human cell threshold. This hierarchy is particularly germane since some reviews are given for studies found across a variety of material classes and biological models.

The characteristics of materials that influence interactions with the gut

The structure, dimensions and surface chemistry of many materials are of significance.

There is no single, standard test substance for MWCNTs. The outer diameter, length distribution, wall number, defect density, catalyst residues and surface oxidation of the commercial and laboratory preparations may be different. Labeling a product "nominal" does not define the properties of the material that will be delivered to the cells. The characterization should describe the material as received and the dispersion used for exposure as sonication, surfactant, protein, salt and pH may be used to affect agglomeration and effective dose. Biological results are found to be different in various laboratories, which may be attributed to material heterogeneity based on reviews (Chetyrkina et al., 2022; Johnston et al., 2010; Mohanta et al., 2019).

In certain biological contexts, the length and rigidity are of significance. Long, stiff fibers are able to escape complete engulfment by phagocytic cells and can also generate persistent tissue interactions, and the diameter and stiffness of fibers have been associated with responses of the mesothelial cells in animal models (Nagai et al., 2011; Poland et al., 2008; Takagi et al., 2008). These results might help guide careful measurements of length and shape, but cannot rule out the possibility that the same fiber paradigm is not true for Caco-2 monolayers. The Caco-2 are epithelial cells and do not have macrophages' phagocytic properties. An interpretation of a mechanism must be expressed as a hypothesis if it is transferred from one cell type to another.

The hydrophobicity, charge, dispersibility, protein-binding and membrane interactions change upon surface functionalization. Carboxylated CNTs, hydroxylated CNTs and polymer-coated materials could be different to pristine MWCNTs. The functional groups can be used to enhance the suspension stability and change the surface that is presented to cells. There is thus a need for chemical and structural characterisation methods to be reported in addition to the biological results (Kim et al., 2014; Ursini et al., 2012, 2014; Wepasnick et al., 2010). For these predictions to be made, the change in surface property due to the modification, and the modification itself, must be measured, and the results should be labelled “modified” with “oxidized” or “functionalized.”

It is important to consider residual metals and other contaminants from the synthesis. Apparent oxidative activity or toxicity may be caused by iron, cobalt, nickel and catalyst-support material. The observed ROS can be from surface of the nanotube, from catalyst residues, from dispersion components or from the interaction between the components of the assay. The presence of contaminants has been shown to impact intracellular ROS responses (Pulskamp et al., 2007) and different types and methods of preparation of carbon-based nanomaterials can affect cellular toxicity (Magrez et al., 2006). The vehicle should then be an appropriate control material, and if there is one, a dispersant-only control should be included, as well as a material characterization report with the elemental composition.

These are related to the dispersion and delivered dose.

The concentration indicated is that the drug is administered at, and not necessarily that which comes into contact with the surface of the cell. MWCNTs can sediment, aggregate, attach themselves to plastic, or be suspended. The delivered dose will vary with the volume of exposure, the geometry of the wells, incubation time, size of the agglomerates, their density and cell layer. Toxicological studies have been conducted in vitro, which have demonstrated that the aggregation state affects the toxicity of CNTs (Wick et al., 2007). This should be accompanied by a description of the method used to disperse the cells and, if possible, an estimate of the amount of mass deposited or attached to the cells.

There can be confounding in the dispersion protocols. Probe sonication can cause damage or contamination to the probe, cause shortening or damage to the nanotubes, and can cause heating. Suspensions may be stabilized by the use of surfactant and/or protein, and may change the biological interface. The sonication power, sonication time, the pulse pattern, cooling, dispersant type and concentration, and preparation to dosing interval should be included in the protocol. A validated dispersion protocol does not ensure a biologically relevant suspension, but allows other investigators to comprehend and replicate the exposure (Wepasnick et al., 2010; Johnston et al., 2010). The nominal mass concentration is also not an absolute index of apparent cytotoxicity in cross-material comparisons as dose and dispersion can alter apparent cytotoxicity (Zhou et al., 2017).

The dosage level chosen should be based on the scientific question. High concentrations can allow to determine the mechanism of the hazards but can also lead to agglomeration, physical coating of the monolayer or to non-specific stress which would not occur if it were considered for oral exposure. However, low dose studies may be difficult to interpret when the dose is too low to be detected by the assay or endotoxin and background contamination are not controlled. There are two types of studies useful, one for hazard identification and one for exposure relevant, but they need to be differentiated on the dose rationale. A concentration series should contain a vehicle control, a positive control for barrier disruption and a range which should not be based on one high dose condition.

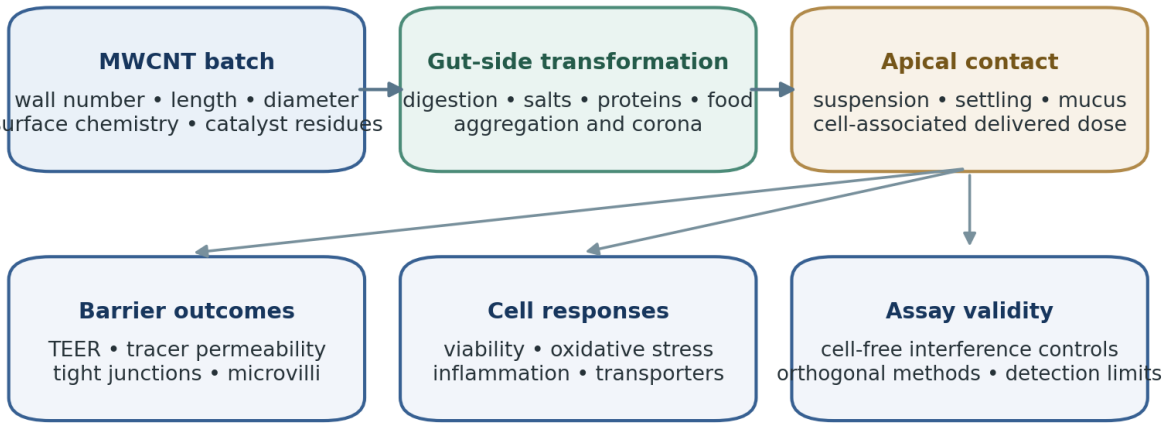
How to transform your voice and use it to impact others.Voice transformation and effective exposure.

If the surface and dispersion state within the cell interface are not the same as those in the as-manufactured material, then this difference will be noted in the oral question. Before reaching the epithelium, a nanotube can be exposed to saliva, the acidic gastric fluid, the bile salts, digestive enzymes, food components and the electrolytes present in the intestinal cavity. The conditions can affect the agglomeration, biomolecules adsorbed, and unsuspended material fraction. A complex matrix, that occurs during digestion, can also have an effect on cell assays without the influence of the nanotube. Gerloff et al. (2013) demonstrated the importance of analyzing particle responses in Caco-2 cells in conjunction with simulated gastrointestinal treatment and cell differentiation state. The gastrointestinal route views also highlight that the chemical transformation is not something separate from biological questions but rather is an integral part of the exposure matrix (Pietroiusti et al., 2017). To demonstrate the ability to correlate material transformation with biological testing with only in vitro degradation studies using gastric agents in the MWCNTs and in vivo testing of the murine intestine, we performed a preliminary study (Masyutin et al., 2015).

There is no direct correlation between an oral dose given to an animal and a nominal concentration of the culture. The administered dose is given by lumen diffusion, will bind to food/mucous membrane, can be excreted and only part will be in contact with the epithelial surface. The dose delivered in vitro is dependent on sedimentation, diffusion, well geometry, depth of the medium and aggregation. The number of fibres, length distribution, surface area and mass may be different exposure indicators for a high aspect ratio material. When there is a variation in dimensions or density of a material panel, it is useful to report more than one metric. If an exposure estimate is not realistic, studies should be marked as "hazard identification" and not imply that these conditions are an exposure to the diet. Although the concentration-response curve is still useful, it is better when used in conjunction with measured dispersion stability, cell-associated mass, or a calculation based on a model to estimate delivery dose.

The results of simulated digestion can be most informative if the research question is: What happens to an ingested preparation after passing through the gastrointestinal tract? Not necessarily required for all mechanistic studies. When the question is posed directly and with no transforming factors (previously, the question would have included the two components of the cell material interaction), the interaction of cell and material can be isolated. In the case of food or oral-carrier situations, it is useful to compare the pristine and digested preparations to detect the effects of changes in digestion on uptake, barrier effects or assay interference. Appropriate digestion blanks and matrix-only controls are essential as the digestive fluids can change the TEER, viability or optical endpoints. This is a separation from intrinsic epithelial hazard versus risk of a product consumed.

Figure 1
From material identity to intestinal response



Note. Original conceptual schematic. Arrows indicate an experimental reasoning path, not a demonstrated causal sequence.

Caco-2 cells as an intestinal barrier model
Strengths and appropriate uses

Caco-2 cells are able to polarize into a single layer of epithelial cells having both apical and basal compartments. They have proven to be suitable for study of intestinal drug absorption (Artursson, 1990; Hidalgo et al., 1989; Hubatsch et al., 2007) due to their permeability and transport properties. The model enables the measurement of transepithelial electrical resistance (TEER), paracellular permeability, cell viability, cell association and selected molecular responses in a human based system of epithelial cells. The model can be used to compare in a controlled way the material type, the state of the surface chemistry, the exposure time and the differentiation state.

Maturity of models should be determined and not assumed. The degree of Caco-2 differentiation is dependent on passage number, cell origin, culture media composition, serum, seeding density and culture time. Good culture practices and the effect of cell and culture factors on barrier properties were described by Natoli et al. (2012) and Sambuy et al. (2005), respectively. TEER is not sufficient to describe the property alone as it is not the same for all instruments and all laboratories and a high resistance does not guarantee all properties of the enterocytes. Baseline permeability, morphology and expression of markers should be reported as well as stability over time.

There are also aspects of the model that are relevant for CNTs that have limitations. The human gut has a mucus layer and microbiota, an immune compartment, fluid flow and tissue architecture that are absent in a monolayer. The expression profile of transporters can be seen in Caco-2 cells, and, however, does only not mimic every region of the small intestine. Responses in a cell line derived from a cancer may be useful to understand cellular mechanism, but should not necessarily be equated with response of healthy intestinal tissue. Epithelial models of the intestine have been used in studies to show how maturity of the cells and the characteristics of the particles influence the measurement of response to nanoparticles (Gerloff et al., 2009, 2013; Abbott Chalew and Schwab, 2013).

The concepts and tools of co-culture and more complex systems are introduced.

Co-cultures of Caco-2 and goblet-like cells (Caco-2/HT29-MTX) can be used to generate mucus-secreting goblet-like cells with a physiologically relevant apical interface that is superior to that of a monoculture. But this will depend on the proportion of the cell types, the length of the culture and the growth conditions. The composition of the mucus layer can influence the availability of particles to the epithelial surface and co-culture composition can also modify barrier permeability. The intestinal models for nanomaterial

testing are more complex as described by Susewind et al. (2016) and Ude et al. (2019) and characterization of human in vitro intestinal systems for hazard assessment was discussed by Gibb et al. (2021).

Not every question will have a better model than a simple one. Useful for questions addressing epithelial mechanisms is monoculture, useful when mucus is important is co-culture, and useful for questions involving inflammation, flow, and/or tissue-level transport is immune-cell co-culture or organ-on-chip approaches. Biological features and experimental variability are added to each model. The necessary research should be done on the simplest model that will test the hypothesis stated and the description of the things this model does not represent. Ritarossi et al. (2025) recently reviewed the variety of intestinal in vitro models employed to study the uptake and crossing of nanoparticles and the requirement for a consistent characterization of them.

The Caco-2 cells are also a specific epithelial lineage and cell culture history. They are derived from a human colorectal adenocarcinoma and when differentiated, gain selected features which resemble the absorptive enterocytes. No regional specialization occurs in the small intestine, including differences in the amount of transporters, or the different luminal environment, in their absence. A conventional insert culture also lacks blood circulation to remove transported material, peristaltic shear and endocrine and neural signals. The model is most useful for questions regarding controlled epithelial contact, permeability and cell autonomous response. It is less effective at estimating whole organism absorption, systemic distribution and/or adaptation over time. These boundaries do not make the system unsuitable, they are an indication of what the system can withstand.

Quality control should be made specific and related to the experiment, not be a boilerplate statement. The acceptance threshold should be justified for this laboratory and this insert format and can only be determined after the baseline TEER has been measured before the exposure. A leaky monolayer in which the resistance reading is acceptable can be picked out by a tracer permeability measure. A positive barrier-disruption control can confirm that the assay reveals a biologically significant disruption of the barrier. The following parameters should be reported as these can influence apparent barrier behavior: Passage number, differentiation interval, seeding density, cell ratio in co-culture and cell geometry of the plates. These controls allow the identification of whether the discrepancies between two studies are due to the MWCNT material or due to a lack of functional similarity between the two (Hidalgo et al., 1989; Natoli et al., 2012; Sambuy et al., 2005).

Although not CNTs, graphene oxide and graphene nanoplatelets are explored in the context of Caco-2/HT29 barrier models to provide a methodological context for the carbon nanomaterials. Domenech et al. (2020) have shown that the interaction between an intestinal barrier model and carbon nanomaterials is affected by the material form. The results of such studies could be used to consider the selection of assays and to interpret the carbon-based particle interference; however, they may not be used as toxicology evidence specific to MWCNTs. The same applies to studies in Caco-2/HT29-MTX cells (Gillois et al., 2021) and three dimensional gastrointestinal models for copper oxide (Ude et al., 2019).

No direct evidence for MWCNT and Caco-2 system
Uptake, viability and structure of the epithelia

The study of Clark et al. (2012) is a direct study of MWCNT-Caco-2. The authors had tested the Caco-2 cells with pristine or oxidized MWCNTs for 24 hours at a nominal concentration of 50 micrograms per milliliter both in the presence and absence of natural organic matter. In those conditions, they did not notice any significant difference in the viability of cells and no internalization within the Caco-2 cells were noticed. They did note some damage to the microvilli that was correlated with the nanotubes. This finding demonstrates the need to not equate “no loss of viability” with “no epithelial effect” as structural features can change, but no acute viability signal is generated. It is not known if all forms, concentrations or repeated exposures of MWCNT are equally non-cytotoxic.

The lack of detection of uptake in a differentiated Caco2 model can be harmonious with a number of possibilities. The nanotubes can be apical, attached to the cell surface, integrated into the mucous or membrane structures, or be below the imaging detection level. Association and internalization of cells is differently sensitive to measurement methods. There must be image controls, orthogonal quantification, if available, and more importantly, procedures that differentiate extracellular material from internalized material in order to make a robust uptake claim. The broader context of the interaction of the CNTs with the cells and the context of the microscopic study is provided by Ponti et al. (2010); however, conclusions concerning the intestine may only be drawn if cell type and assay are matched.

Predictive changes in protein and mRNA levels

Lai et al. (2013), investigated the functionalized CNTs in the Caco-2/HT29-MTX co-culture. Carboxylated SWCNTs, MWCNTs and polymer-wrapped MWCNTs were all included in the materials. The study employed quantitative proteomic analysis and revealed material-specific expressions (e.g. dose-related and dispersion

stability-related differences). It did not imply that all protein responses were not found, as there was no overt cytotoxicity. The results indicate that molecular response profiles are able to identify effects that would be missed by conventional viability tests. They also demonstrate that in the case of surface chemistry and colloidal behavior being different, the term “MWCNT exposure” is too general.

Tilton et al. (2014) compared the responses in three types of human cells (caco-2/HT29-MTX intestinal cells) after exposure to MWCNTs and TiO2 nanobelts. Early and late responses were taken and low and high nominal concentrations were used. The transcriptomic and proteomic profiles varied across the different cell types and exposure times. This study corroborates the idea that it is not possible to describe all biological reactions to the same nanomaterial with one pathway or cell type. More recently, Basak et al. (2016) used mathematical nanotoxicoproteomic analysis to intestinal co-culture data for MWCNT and nanobelts of TiO2. These studies illustrate the merit and sophistication of molecular endpoints, and raise the question of the reliability of short term in vitro signatures as predictors of oral risk in humans.

The designs of these molecular studies are also a window on the amount of direct evidence. Lai et al. (2013) studied a number of functionalized CNT preparations in a co-culture with mucus and identified material-dependent patterns via proteomics analysis. Having it in both forms (carboxylated and polymer-wrapped MWCNTs) is beneficial since each one is in a different surface state and colloidal form, which does not give a universal MWCNT signature. Tilton et al. (2014) sampled various cell types and times following exposure to MWCNTs, demonstrating that cell response after exposure varies by cell type and duration of exposure. The subsequent mathematical analysis done by Basak et al. (2016) is useful as an interpretation of the proteomic patterns and should not be considered as a replication of each observation but rather related experiments. When assessing the size of the evidence base it is important to distinguish between a new analysis and a new biological experiment.

The same "word of advice" applies to the transporter study. ABCB1 and ABCC4 are functional transporters and the changes reported warrant examination of substrate efflux in differentiated intestinal epithelium. However, the expression of the transporters is variable to culture and a cancer-derived cell line may have a different basal signaling. Follow up studies should indicate if the activity is changing per viable cell, if possible, test more than one substrate, and have a recovery or wash out period. When an MWCNT preparation is being considered for oral delivery, experiments must be conducted on both the loaded preparation and the unloaded to assess release kinetics and determine the function of the transporter. These are equivalent to designs that are able to differentiate between desired drug transport and unintended changes in epithelial pharmacology.

Discuss the role of transporters and their relevance in drug action and disease.

Wang et al. 2015 reported that transport activity of some ATP-binding cassette transporters (ABCC4/MRP4 and ABCB1/P-glycoprotein) were inhibited in Caco-2 cells after exposure to MWCNTs, and that the expression of these transporters was also decreased. The study correlated the change with lowered expression of c-Myc and discovered that the re-expression of the c-Myc reversed transporter impacts.The study correlated the change with lowered c-Myc expression and discovered that re-expression of c-Myc changed transporter impacts. The result is of special relevance because cells typically used for the evaluation of drug transport, the Caco-2 cells, were used. It also makes for a more complicated “carrier” story: a nanotube designed to deliver a therapeutic agent might be able to change the normal transport mechanisms, cell signaling or efflux of drugs.

Results of the transporter should be interpreted in the context of the model. Caco-2 cells are cancer derived and therefore, expression of transporters in an adenocarcinoma line might not be the same as in healthy small-intestinal epithelium. The experiment is used to determine a mechanistic interaction and a potential drug delivery issue but is not used to quantify the clinical effect of oral MWCNT exposure. The barrier integrity and transporter function should thus be determined in drug delivery studies performed with functionalized carbon nanomaterials, in addition to the evaluation of the performance of the transported drug (Coyuco et al., 2011; Garriga et al., 2020).

Studies of intestinal cells that are related to this research.Studies on intestinal cells, which are related to this.

There are several studies that employ SWCNTs instead of MWCNTs for the Caco-2 study. Carboxyl-functionalized SWCNTs were investigated by Jos et al. (2009) in differentiated and undifferentiated Caco-2 cells, which showed a differentiation-dependent toxicity. The same cell line was used in a study of oxidative-stress responses to carboxylated SWCNTs by Pichardo et al. (2012). Chronic exposure to SWCNTs by low dosages has been reported to impact bacterial invasion and inflammation in Caco-2 cells (Chen et al. 2015). The studies are applicable to potential mechanisms and to which assays to use, but they are not able to establish the response to MWCNTs, which vary in size and number of walls.

A functionalized carbon nanomaterial, such as a SWCNT formulation or fullerene materials, was studied

for possible oral drug delivery in Caco-2 cells by Coyuco et al (2011). The paper is useful for uptake and transport ways, but it has a focus on designed delivery systems as opposed to hazard identification of unformulated MWCNTs. Garriga et al. (2020) studied carbon nanomaterials with Caco-2 and MCF-7 cells from a delivery perspective. These studies suggest a potential use and biological activity in carbon nanomaterials, but the separation of the product design, loading, release of the carrier, and exposure to free material should be done experimentally.

Intestinal evidence found in cells other than Caco-2

Lahiani et al. (2019) evaluated the human intestinal epithelial cell model T-84 with and without MWCNT residues in tomato fruits, and a human fecal suspension system. They showed no significant change in transepithelial resistance or in certain gene expressions. This is applicable to food-chain exposure, but is not a Caco-2 experiment. The cell types T-84 cells do not have the same characteristics as Caco-2 cells and the low residue level corresponds to a particular plant-growth and exposure scenario. The outcome is meant to be used to infer about the tested material and condition, but not to infer that the presence of MWCNTs in the intestine generally is harmless to intestinal epithelia.

Golokhvast et al. (2013) followed the orally administered MWCNTs in mice for 6 days and reported effects in the gastrointestinal mucosal tissues. In mice, after repeated oral ingestion, Masyutin et al. (2016) later observed necrotic foci in the small intestine, associated with the presence of multiwalled carbon nanotubules. These animal based processes at the level of the dose, material and tissue are different from an in vitro Caco-2 system. The studies do, however, highlight the importance of specific investigation of the oral route and repeated exposures. Recently, Lv et al. (2026) reported intestinal histopathology, the response of genes related to apoptosis, the response of genes related to tight junctions, and changes in the microbiome-metabolites in common carp when exposed to MWCNTs. The results of this fish study expand the ecological and comparative evidence, but it can't be directly applied to predicting epithelial outcomes in humans.

Selected intestinal and Caco-2 related CNT studies are summarized in Table 1. It differentiates the material type and model, allowing SWCNT, T-84, animal, and Caco-2 evidence to be identified.

Table 1
Representative CNT evidence relevant to intestinal models

Evidence group	Study and model	Main finding	Interpretive limit
Direct MWCNT	Clark et al. (2012); Caco-2	At 50 µg/mL for 24 h, no marked viability loss or uptake was reported; microvilli damage was observed.	One material preparation and acute exposure; assay sensitivity limits uptake claims.
Direct MWCNT	Lai et al. (2013); Caco-2/HT29-MTX	Functionalized MWCNTs produced material-dependent proteomic responses, including responses without overt cytotoxicity.	Mucus co-culture and functionalized materials; not a universal pristine-MWCNT response.
Direct MWCNT	Wang et al. (2015); Caco-2	MWCNT exposure reduced selected ABC transporter expression and transport activity, with c-Myc implicated.	Cancer-derived model; clinical oral exposure and healthy tissue are not inferred.
Direct MWCNT	Tilton et al. (2014); Caco-2/HT29-MTX	At 10 and 100 µg/mL, transcriptomic/proteomic responses varied by cell type and time.	Multi-cell comparison; nominal concentration is not a delivered-dose estimate.
Reanalysis	Basak et al. (2016); intestinal co-culture proteomics	Mathematical analysis characterized response patterns from MWCNT and TiO_2 nanobelt exposure.	Related dataset analysis; not an independent biological replication.

Evidence group	Study and model	Main finding	Interpretive limit
Related SWCNT	Jos et al. (2009); Pichardo et al. (2012); Caco-2	Carboxylated SWCNT studies reported culture-state-dependent cytotoxicity and oxidative responses.	Single-walled material; useful for endpoint design, not an MWCNT effect estimate.
Related SWCNT	Chen et al. (2015); Caco-2 with bacterial challenge	Low-dose SWCNT treatment altered bacterial invasion and epithelial inflammatory outcomes.	Infection context and SWCNT material differ from MWCNT hazard testing.
Other human gut model	Lahiani et al. (2019); T-84 and fecal systems	Tomato-derived residue and 0.001 µg/mL CNT did not significantly alter selected barrier/gene measures; 10 µg/mL produced cell-associated effects.	T-84 is not Caco-2; food-chain residue scenario is specific.
Oral animal	Golokhvast et al. (2013); mouse, 6-day oral exposure	MWCNT exposure was associated with changes in gastrointestinal mucosal tissues.	High-dose animal context; cannot define a Caco-2 or human threshold.
Oral animal	Masyutin et al. (2015, 2016); mouse intestine	Gastric-agent transformation and repeated oral exposure were studied; the 2016 study reported intestinal lesions.	Conference report and animal dosing; limited direct comparability to cell culture.
Adjacent species/material	Chen et al. (2018); SWCNT in mice; Lv et al. (2026); MWCNT in fish	Oral CNT studies identify barrier, inflammatory, microbiome, or intestinal tissue endpoints for follow-up.	Different wall number or species; hypothesis-generating context only.

Note. Rows distinguish direct MWCNT–Caco-2 evidence from adjacent material, model, and species evidence. Study findings are concise summaries; see cited primary articles for methods.

Mechanistic interpretation

Barrier integrity and tight junctions

Barrier integrity is a functional outcome; it is NOT cell survival. TEER is a parameter that measures the ionic conductance through the monolayer and provides information on the changes in barrier function but not the cause of the change. An alternate assessment is the tracer permeability through the paracellular pathway. ZO-1, occludin and claudins can be immunostained/imaged to demonstrate changes in the organization of the junctions, and ultrastructural analysis can reveal the presence of microvilli or membrane damage. Changes in epithelial permeability could be missed by a study looking at just MTT or another metabolic viability measure.

The barrier can interact with MWCNTs in a variety of ways, such as by direct contact with the cell membrane, by disrupting cell-cell interactions, by modifying cytoskeletal structure and by causing oxidative and inflammatory signaling. Until the endpoint(s) of the mechanism can be measured in the same experiment, it is only a hypothesis. Assay interference, cell death, and integrity of the monolayer should be determined as causes of a decrease in TEER. On the other hand, changes in transporter function may occur without affecting the stability of the TEER.However, gene expression, subtle molecular stress, microvilli morphology, and changes in transporter function may occur without changes in the stability of the TEER. The Clark et al. (2012) observation of microvilli changes, but without significant loss of viability, is a good example of endpoint dependence.

Oxidative stress and inflammatory signaling mechanisms.

One of the suggested mechanisms of CNT's toxicity is oxidation. The presence of CNT surfaces and related residues of the catalysts can increase the production of ROS, and cells could deal with this by using antioxidant pathways, by undergoing lipid peroxidation, and by altering redox-sensitive signaling pathways. The fluorescent ROS assays, however, may be skewed by light absorption, light scattering, fluorescent autoabsorption of assay reagents, and/or autofluorescence of the

nanotubes. A positive signal should be accompanied by controls which assess the interference of nanoparticles in the assays, and with orthogonal measures, if possible, such as antioxidant depletion, oxidative-damage products or gene and protein responses.

MWCNTs have been reported to induce oxidative stress and cytotoxicity in other cell types, outside of the intestine. It can be seen in the case of the pulmonary epithelium (Cavallo et al., 2012; Hirano et al., 2010; Srivastava et al., 2011; Ursini et al., 2012, 2014). Shvedova et al. (2009, 2012) reviewed the mechanisms in the lungs and the contribution of oxidative stress. All of these findings would help validate measuring redox responses in intestinal studies, but they do not show that the Caco-2 cells respond the same way. The outcome may vary depending on the cell-specific dose, uptake, antioxidant capacity, and conditions of the cultures.

There needs to be model awareness for inflammatory readouts too. Selected inflammatory mediators can be expressed in caco-2 monocultures, but do not include the immune-cell networks which define mucosal inflammation. An epithelial signaling (cytokine change in a monoculture) is not a complete inflammatory phenotype. If the hypothesis is related to immunotoxicity, then co-culture with immune cells or complementary immune assays may be required. Tilton et al., 2014, and the reviews of CNTs by others emphasize that there are significant differences between the different models, especially epithelial vs. immune.

The cell association, uptake and assay artifacts are removed from the data.

For high aspect ratio materials it is important to know whether surface association or internalization occurred. Localization is usually detected by electron microscopy which may sample only a few cells and may be influenced by preparations artefacts. If the material can be labeled, without altering its properties, or with a label that has been carefully validated, then confocal microscopy may be useful. ICP methods can detect the elements of the catalyst metals used in carbon nanotubes, instead of the carbon nanotubes themselves. There is no single method that is adequate for all experiments. The method choice should be based on the biological question and the claims should be in line with the detection capability.

Nanotubes can also affect the readout of the assays due to binding, absorption, quenching, scattering, etc. of the dyes. Absorbent-based assays (such as MTT or WST-1) may give erroneous results of either apparent viability or the appearance of viability when particles interact with the assay reagent or optical detection. These can help to minimize this type of risk: cell-free assay controls, particle-only blanks, washing steps, and complementarity of the endpoints. The need for characterization of materials and validation of assays for meaningful comparisons is highlighted when reviewing toxicity and nanotoxicoproteomics research carried out by CNT (Chetyrkina et al., 2022; Francis & Devasena, 2018; Kim et al., 2014; Magrez et al., 2006).

PWPs involves transporters, membrane function and microbiota.PWPs entails transporters, membrane function and microbiota.

The results of the transporter reported by Wang et al. (2015) suggest a mechanism different from barrier resistance. ABCB1 and ABCC4 regulate efflux of a variety of compounds across cells. The changes in expression and/or transport activity may impact the absorption of drugs, the exposure within the cell and the interaction with drugs. Follow up work should be able to differentiate direct interaction with the membrane from transcription regulation and should compare several transporter substrates and/or inhibitors. This is particular when MWCNTs are taken as oral drug carriers.

Ingested nanomaterials can alter the biological context by impacting the surface coronas or aggregation or by altering local metabolites in the gut microbiota, while nanomaterials can also impact the viability of gut microbes and/or community composition. Such as complex bidirectional interactions between nanomaterials and the gut environment, which have been described in reviews (Fröhlich & Fröhlich, 2016; Qiao et al., 2024; Utembe et al., 2022). It is not possible from a monoculture of Caco-2 to address these interactions. Microbiome questions may require in vitro fecal fermentation or epithelial-microbiota co-culture studies, or animal studies, but all have their own validity limits. Intestinal evidence should be interpreted based on the route of exposure, species, dose, and model for MWCNT exposure in plants, mice, and fish (Lahiani et al., 2019; Lv et al., 2026; Masyutin et al., 2016).

Studies have found differing results, why?

Disagreements between the different studies of the CNT do not necessarily constitute a contradiction. These endpoints reflect different biological processes, and may not be detectable in one study but in another even though there is no acute loss of viability. Another source of the variation is material differences. The surfaces and the dispersion state of pristine, oxidized, carboxylated, hydroxylated and polymer-wrapped MWCNTs are different. It is important to note that a result obtained from one preparation cannot be extrapolated to all MWCNTs (these claims are made by Lai et al., 2013; Kim et al., 2014; Wepasnick et al., 2010).

The phenotype of the cells is also important. Undifferentiated cultures made with Caco-2 cells are not as permeable as differentiated monolayers. Caco-2/HT29-MTX co-cultures contain a mucus coating which can limit or alter particle access. Responses of transporters and junctions may vary with different cell lines (e.g., T-84). The effects of culture conditions on Caco-2 characteristics have been known for a long time

(Natoli et al. 2012; Sambuy et al. 2005). Cell source, passage, culture length, seeding ratio, medium and pre-exposure barrier criteria should be reported in studies.

The effective dose can vary with exposure conditions. The changes in agglomeration and sedimentation of the particles are brought on by sonication, dispersants, serum proteins, ionic strength, and pH. The digestive process may change the properties of a nanomaterial prior to contact with the epithelial layer. Gerloff et al. (2013) demonstrated that particle-response measurements in differentiated Caco-2 cells are affected by simulated gastrointestinal conditions and differentiation state of the cells. Therefore, pre-digestion material must be distinguished from pristine stock material and written exposure medium description given in detail.

Lastly, there are scale differences between route and biological. Pulmonary effects and concerns related to fiber have been observed in animals in studies of inhalation exposure to MWCNTs (Ma-Hock et al., 2009; Muller et al., 2005; Poland et al., 2008; Ryman-Rasmussen et al., 2009). The results obtained justify occupational and environmental controls, but cannot be used to determine the risk from oral exposure. The particle dose delivered to the gut following mucociliary clearance can be altered and can be quite different to that delivered in an oral bolus. The gastrointestinal consensus literature explicitly warns of a change in exposure route changing the material that reaches the gastrointestinal tract (Pietroiusti et al., 2017).

The experimental design and reporting representative examples are shown below:

The first step in an MWCNT-Caco-2 study should be to establish the exposure scenario. Is it to find intrinsic epithelial hazard, to evaluate an oral carrier, to test environmental/food exposure or compare functionalized materials? The material panel, dose range, exposure duration and model are determined by the question. A wide range of concentrations may be required for hazard identification studies and exposure-relevant studies should demonstrate the justification of concentrations by an exposure model and not give the impression of realism if a concentration is low.

Characteristics of the MWCNT batch that should be evaluated include length and diameter distribution, surface area (if available), surface chemistry, zeta potential in the exposure medium, agglomeration state, elemental impurities, and endotoxin. Dispersions should be repeatable. Only add a pristine material and a functionalized form of it, if this comparison supports the hypothesis. Include dispersant-only controls when using dispersants. Describe dispersion as a function of time after dosing and after a period of exposure when there is a possibility of settling/aggregation. These practices follow the general nanotoxicology guidelines and CNT characterization reviews (Johnston et al., 2010; Wepasnick et al., 2010).

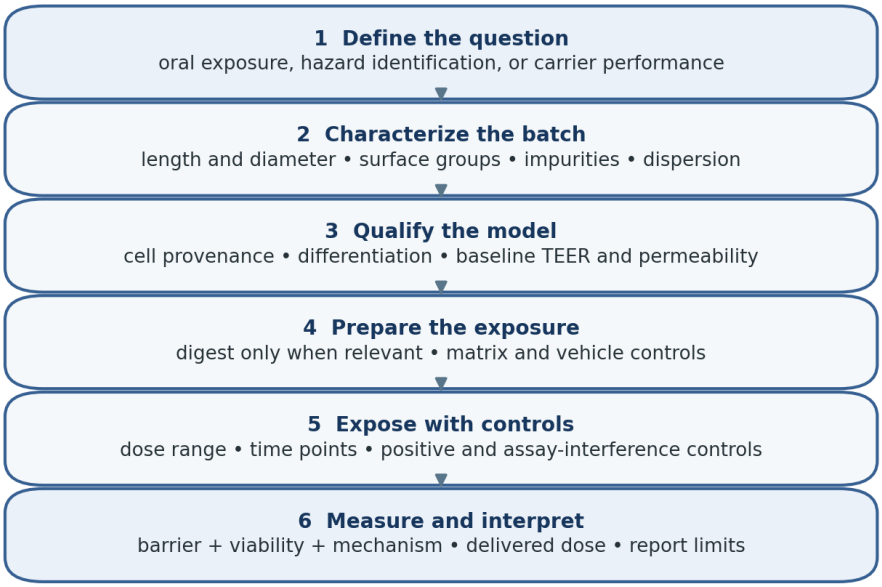
Cells should be authenticated and should be tested for the presence of mycoplasma. Passage number, seeding density, days after seeding, media, serum and differentiation conditions should be reported. Prior to exposure, baseline TEER and permeability measures should be used to ensure integrity of the monolayers. If there is a relevance for mucus, use a co-culture with Caco-2/HT29-MTX, which was incorporated with a ratio, and check for production of mucus. Where the objective is mechanism isolation, a monoculture is the proper choice, and only conclusions regarding the response of the epithelial cells from the monoculture should be drawn. Additional, more complex, intestinal systems should be introduced only when they are a test of a specific biological feature (Gibb et al., 2021; Ritarossi et al., 2025; Susewind et al., 2016).

Exposure should be repeated doses as well as multiple concentrations, at least two time exposure if applicable. Report administered concentration and an estimate of delivered dose/cell associated material. A simulated digestion or if justified, a bioaccessibility step, may be appropriate for oral exposure questions. Agglomeration and protein adsorption can be influenced by the digest matrix and the transformed material should be measured for this reason. It is important that the difference between undigested MWCNT and a digestion transformed suspension is always apparent during the analysis.

A core endpoint panel should consist of TEER, tracer permeability, viability and LDH release as well as microscopy of the monolayer. Based on the hypothesis, add tight-junction markers, transporter activity, oxidative stress, inflammatory mediators and cell-associated CNT measurements. A minimum of one viability assay should be tested using particle interference. Use cell-free nanoparticle-plus-reagent controls, barrier disruption and oxidative stress positive controls and suitable vehicle controls. Wherever possible use blinded image scoring and report independent biological replicates separately from technical replicates.

Reporting should reflect the experimental design; statistical reporting should be consistent with the experimental design. Predefine the primary outcome, explain sample size or give a rationale, report individual data points and effect sizes, and report the model used for repeated measures or multiple doses. Nonsignificant difference should not be considered as evidence of equivalence or safety unless equivalence testing was a study's primary objective. If possible, include raw data and analysis scripts. Tables and figures will be distinguished as independent experiments, technical wells, and exposure batches.

Figure 2
Recommended workflow for MWCNT–Caco-2 testing



Note. Original workflow schematic. Simulated digestion is included when it matches the oral exposure question.

Table 2 gives a compact reporting checklist. Its purpose is not to impose a single assay protocol but to identify minimum details needed to interpret MWCNT-Caco-2 comparisons.

Table 2
Minimum reporting checklist for intestinal MWCNT studies

Domain	Minimum reporting items	Why it matters
Material identity	Supplier/batch; wall type; length/diameter distributions; surface chemistry; catalyst residues; endotoxin.	MWCNT preparations differ, and impurities or geometry can change apparent biological activity.
Dispersion and dose	Stock recipe; sonication energy/time; dispersant; medium; nominal concentration; delivered-dose estimate; stability over exposure.	Nominal mass alone does not show how much material contacts the monolayer.
Caco-2 model	Cell source/authentication; passage; seeding density; differentiation time; medium; co-culture ratio; barrier acceptance criteria.	Model maturity and culture conditions alter barrier and transporter phenotype.
Oral relevance	Exposure scenario; digestion or food matrix where justified; matched matrix blanks; transformation characterization.	Gastrointestinal processing can change the material and the assay matrix.
Barrier function	Baseline and post-exposure TEER plus tracer permeability; positive barrier-disruption control; junction/microvilli imaging when relevant.	Viability alone cannot establish preserved epithelial function.
Assay validity	Particle-only and cell-free reagent controls; orthogonal viability/ROS methods; image or quantification limits for uptake.	CNTs can absorb, scatter, quench, or adsorb assay components.

Domain	Minimum reporting items	Why it matters
Analysis and transparency	Independent biological replicates; prespecified primary endpoint; individual data/effect sizes; code/data; limitations.	Makes negative results interpretable and findings easier to reproduce.

Note. This checklist supports interpretation and reproducibility; it does not prescribe one universal assay protocol.

Evidence synthesis and implications

There are a number of conclusions with varying degrees of support based on the direct evidence from Caco-2. In the first study, there was no significant change in acute viability and no detectable uptake for the MWCNT preparation and conditions examined, although a microvilli effect was observed (Clark et al., 2012). Secondly, molecular responses have been demonstrated to be different depending on the functionalization, concentration, and exposure time of MWCNTs in intestinal co-culture studies (Basak et al., 2016; Lai et al., 2013; Tilton et al., 2014). Third, selective drug transporters may be affected by the presence of MWCNT in the Caco-2 cells (Wang et al., 2015). These results compare well because they measure different aspects, employ different materials and designs.

It is very helpful to break up three questions when interpreting the evidence. Is the tested preparation a cell killer and/or a cell detacher? Does it alter an epithelial function like the paracellular permeability, structure of the microvilli or the structure of efflux transport? Does it cause a molecular response that continues or foreshadow tissue damage? The existing direct literature contains some information for each question, but no one study exists that covers all questions. All the above contribute morphology, acute viability observations; all the above contribute molecular profiles; all the above contribute transporter expression and activity. These are not mutually exclusive. So, if one of the assays is not cytotoxic, it is not enough to cancel the compromised barrier; if a protein is found to have a signal, this does not constitute a compromised barrier.

Thus there is a stronger inference for the specific experimental claim and weaker for broad hazard claims. For instance, some MWCNT preparations were not observed to cause significant cell death of the Caco-2 cell under certain acute conditions. It also endorses the report that MWCNT exposures could alter specific cellular proteins and transporters in specific Caco-2-based experiments. It is not possible to provide an overall threshold below which all MWCNTs are safe, nor to estimate the human health risk from MWCNTs. There are uncertainties still that should be addressed such as material characterization, repeated exposure, mucus and digestion effects and healthy-tissue validation. This is a more valuable qualified conclusion for the safety assessor and formulation scientist than a simple yes/no statement.

The literature of the CNT-Caco-2 is of interest for comparison, but should be divided by formulation and wall number. The responses of differentiated and undifferentiated cultures can be different, and oxidative and inflammatory endpoints can be dose-responsive (Chen et al., 2015; Jos et al., 2009; Pichardo et al., 2012). Carbon nanomaterial delivery papers discuss the role of the carrier, the uptake, and the effects of the nanomaterials in cells, but the formulations could be designed to maximize the bioavailability of the nanotechnology (Coyuco et al., 2011; Garriga et al., 2020). These papers do not provide direct estimates of MWCNT hazards, but provide support for experimental design and mechanism hypotheses.

There is mixed evidence regarding the intestinal safety of the evidence around Caco-2. For the T-84 study with tomato residues containing MWCNT, no barrier change was observed at the concentration of MWCNT residues left in the wall (Lahiani et al., 2019). Gut effects are reported in mouse and fish studies under the specific dosing conditions used in the studies (Lv et al., 2026; Masyutin et al., 2016). The evidence of the impact of nanomaterials on gut microbiota varies according to the nanomaterial type, dose, species, exposure time, and study quality (Utembe et al., 2022). There may be no single source that can bridge the gap between nominal in vitro exposure and human intestinal risk.

There is no clear evidence to conclude that MWCNTs are not safe or uniformly toxic to intestinal cells. It can be applied in a conditional way: the biological response is influenced by the type of material, the dose of material delivered, the culture phenotype and the type of end point measured. Acute viability should be one of a number of measures. A strong safety assessment should determine if the substance reaches the epithelial surface, if the barrier is intact, if there is a change in transporter activity and if any observed signals are affected by the assay-interference controls.

With biomedical applications, the concern is not just whether there is no loss of cell viability in Caco-2. An oral carrier should maintain barrier function, not disrupt the carrier in an unexpected manner, deliver its payload at the right time, and cause minimal stress to the epithelium that lines the oral cavity. A substance that doesn't affect it in one short-term assay can be affecting it in other assays. On the other hand, molecular response doesn't necessarily indicate tissue damage. The benefit-risk assessment should include a comparison of carrier-only, free-drug, loaded-carrier, and vehicle conditions

and the use of an appropriate barrier model for the planned use.

Research priorities

The first is to increase work with the Caco-2 cell and to create a common MWCNT panel to facilitate this. Ideally, at least one well-characterised pristine MWCNT and one specified functionalised MWCNT should be included in the studies and surface properties and length distributions should be measured. Laboratories should report in common units, and describe dispersion, delivered dose and cell associated material. This would help to establish if the variation is primarily due to material property or culture and/or assay differences.

Second, when comparing models within the same study, the focus is on the similarity or dissimilarity of the models. The same MWCNT dispersion might be applied to a differentiated Caco-2 monoculture, a Caco-2/HT29-MTX co-culture and a more complex epithelial-immune system. A comparison between such different types of head-to-head comparisons would reveal which effects hold true through increasing levels of biological complexity. They would also expose the changes in particle access and/or response to the immune system and/or mucus. Dose metrics and endpoint panel should be the same when comparing models.

Measuring function in concert with molecular response is the third priority. Where possible, these four measures (TEER, tracer permeability, morphology of microvilli, localization of tight junctions, activity of transporters, viability, and redox markers) should be used together. Pathways can be identified using omics methods, but should be linked to functional outcomes and confirmed using targeted assays. Time-course designs need to identify early response response from persistent injury or adaptation. Repeated exposure is important as oral exposure can be repeated and not a single bolus.

The fourth priority is to relate in vitro results to the exposure context. MWCNTs could undergo changes prior to or during interaction with the epithelial barrier, including digestion, alteration of the diet, mucus, and microbiota. The intent is not to make an entire gut in one assay - it is to get a handle on which transformations are relevant for a specific risk question. An exposure-informed model should predict the percent of material that is suspended, sediments, attaches to the mucus layer, or that reaches the cell surface.

Standardization and transparency come as the fifth priority. Common minimum reporting requirements should include: material characterization; cell model provenance; acceptance criteria for barrier; dose and dispersion; assay-interference controls; biological replication; raw data; and limitations. The systematic and scoping reviews of both CNT toxicity and intestinal models of nanoparticles highlight the need for more characterization and common protocols for cross-study synthesis (Chetyrkina et al., 2022; Ritarossi et al., 2025; Utembe et al., 2022). These practices would lead to the ability to interpret negative results and more easily replicate positive results.

CONCLUSION

The literature is small but informative research on the MWCNT-Caco-2. Direct studies show that under certain conditions, there is a limited uptake or viability change, molecular responses in co-cultures are material specific, and changes in drug efflux transporter expression. SWCNT, T-84, animal, pulmonary, and gut-microbiota studies that are adjacent, but ask unique questions, extend the mechanistic context. It is important to not combine these evidence types together.

Future research should include characterisation and quantification of exposure more realistically, verification of the maturity of the barriers of the Caco-2 cells, testing of endpoints other than cell viability, and control of interference from the nanoparticles in the assays. Caco-2 models are helpful when limits are specified, and when results are provided with appropriate co-culture or in vivo evidence. The literature reviewed indicates careful exposure-specific testing and does not warrant a claim of universal safety and/or toxicity of MWCNTs at the intestinal barrie.

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