

Material-Mediated Pyrogens in Medical Devices: Myth or Reality?

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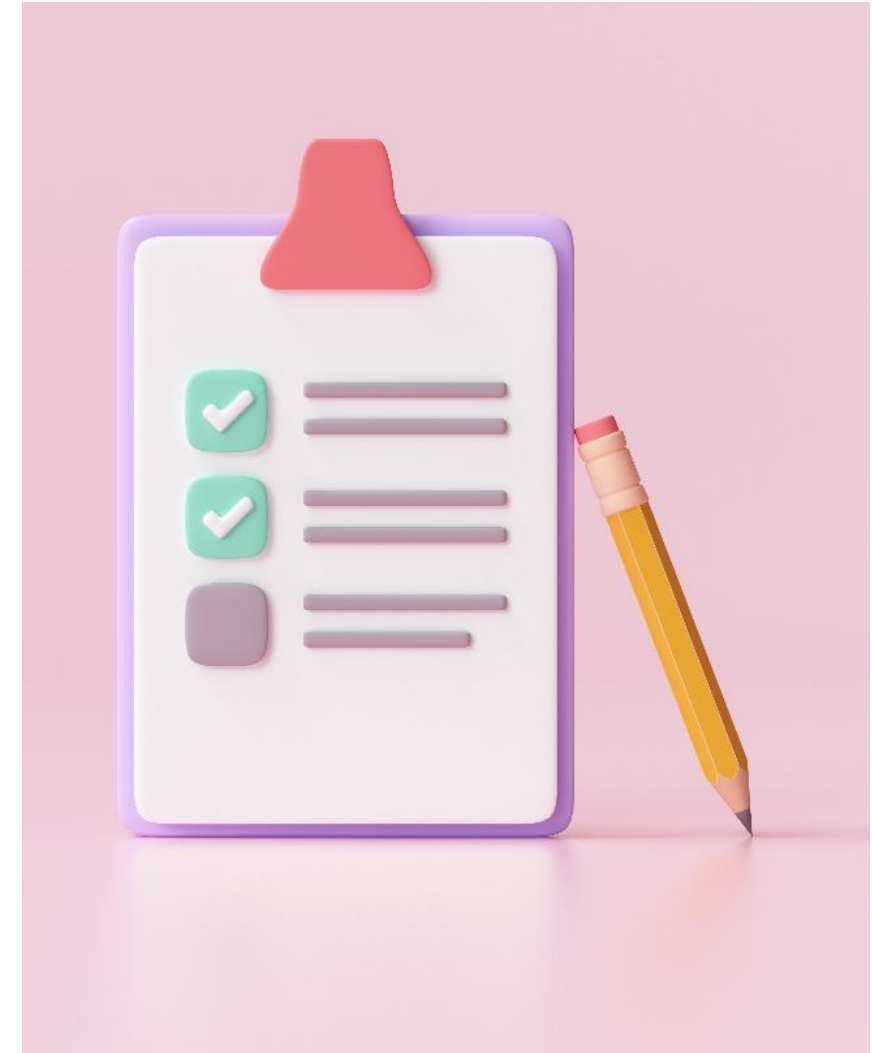
Medtronic

The presenter has no conflicts of interest.

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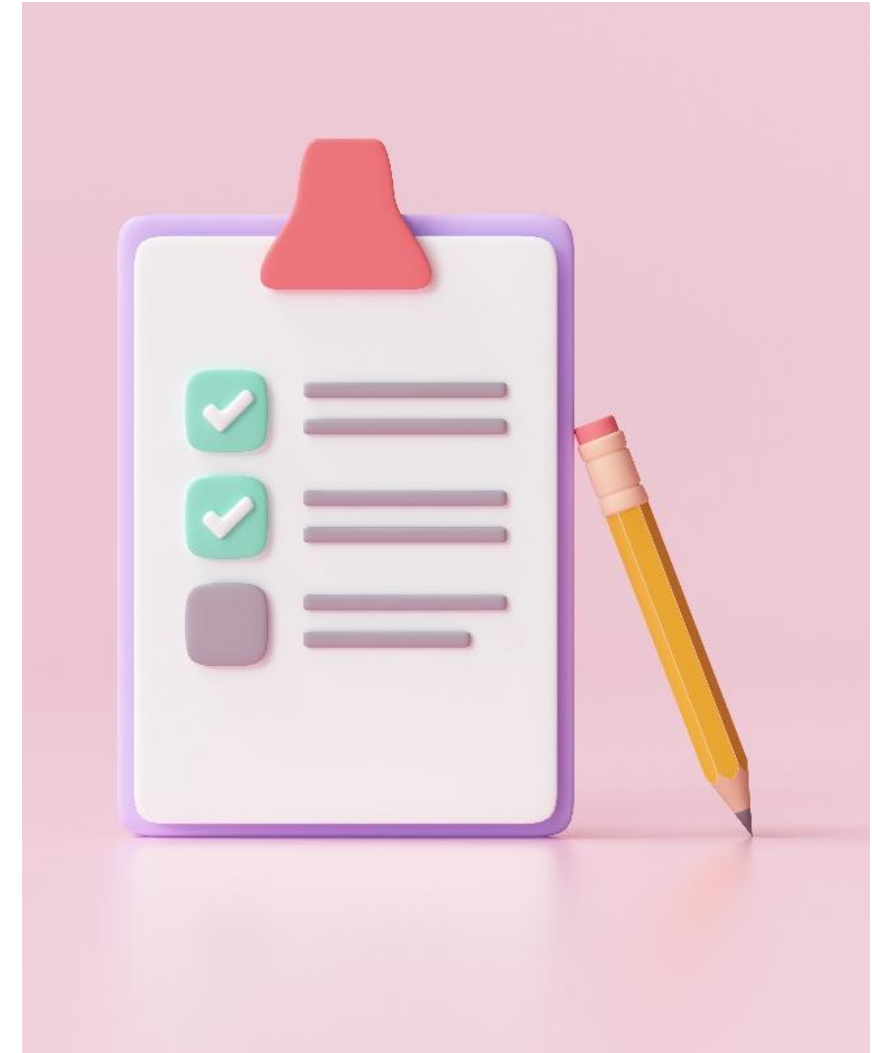
Outline

- Main Points
- Background
- Pyrogenicity *versus* Thermogenesis
- Literature Search Methods and Results
- Data Mining Methods of Results
- Summary and Recommendations



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Material-Mediated Pyrogens in Medical Devices



Review Article

Material-Mediated Pyrogens in Medical Devices: Applicability of the *In Vitro* Monocyte Activation Test

Lindsey K. Borton¹ and Kelly P. Coleman²

<https://www.altex.org/index.php/altex/article/view/1027>

Review Article

Material-Mediated Pyrogens in Medical Devices: Myth or Reality?

Lindsey K. Borton¹ and Kelly P. Coleman²

<https://www.altex.org/index.php/altex/article/view/2977>

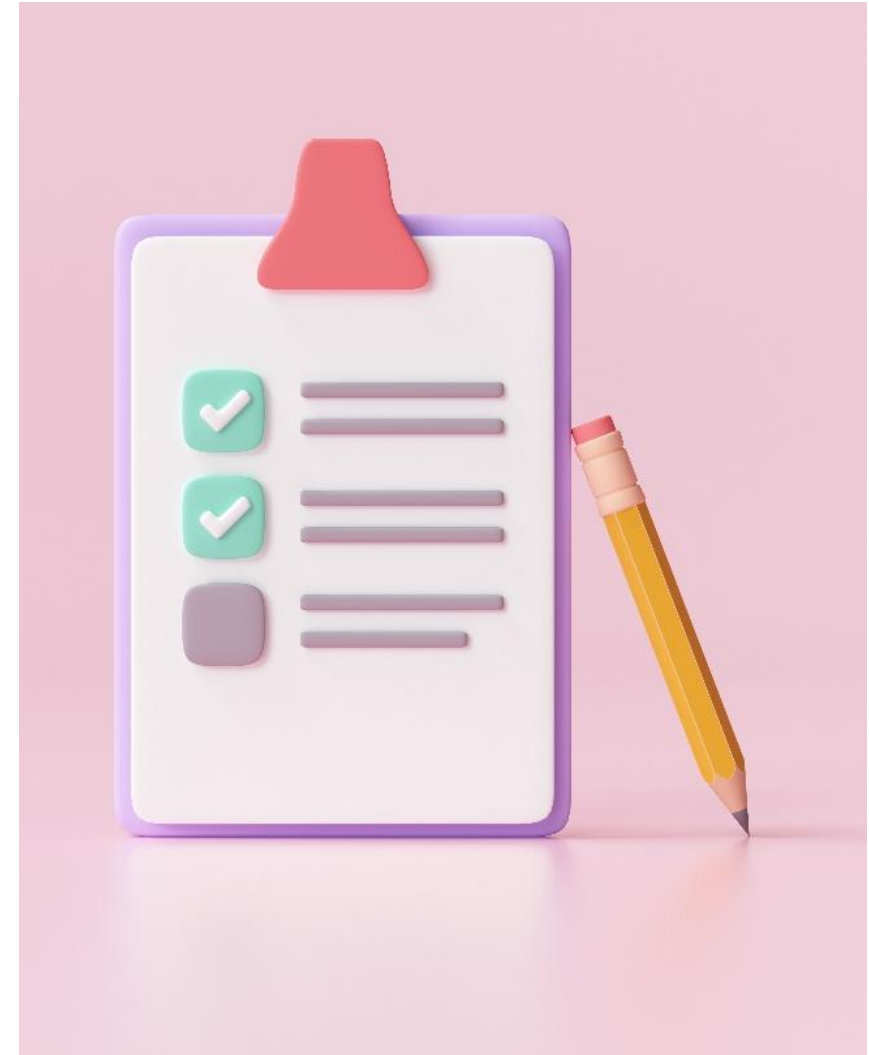


Main Points

- The rabbit pyrogen test (RPT) is inadequate and should be immediately retired.
- Material-Mediated Pyrogens (MMPs) are either: "biological pyrogens" or "chemical thermogens." Accurately identifying what you are looking for is key to supporting medical device safety.
- Based on our data:
 - ✓ Biological Pyrogens should be controlled through manufacturing process or routine Monocyte Activation Testing (MAT)
 - ✓ Chemical Thermogens are extremely rare and do not pose a risk to patients

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Medical Device Biological Safety Assessment

- International Standards Organization (ISO) 10993 is a series of standards that provide guiding principles and frameworks for the evaluation of medical device safety
- Currently, ISO 10993-11 Annex G requires a rabbit pyrogen test (RPT) when there is insufficient data to support device safety for nearly all devices
- There's no official definition of "material-mediated" pyrogen (MMP)
- The rabbit pyrogen test (RPT) is the gold standard for detection of "MMPs"



Pyrogen Testing Standards and Guidance

ISO 10993-1:2025, Section 6.5.10.5, “Material-mediated Pyrogenicity”

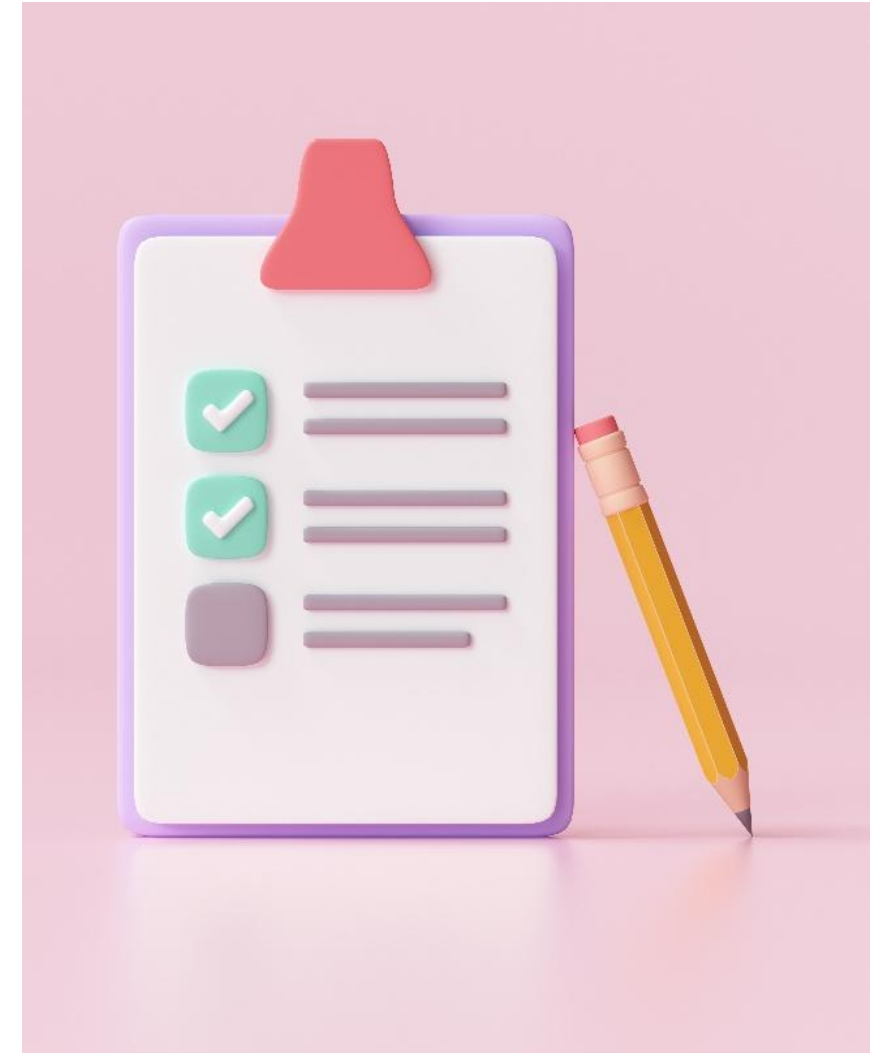
- *“In many cases, it can be sufficient to present a rationale that:*
 - *The **composition** of the finished medical device (including **processing aids** and **manufacturing contaminants**) does not present a significant biological risk of material-mediated pyrogenicity...”*



The information in this presentation could be helpful when conducting a pyrogen risk assessment ...

Outline

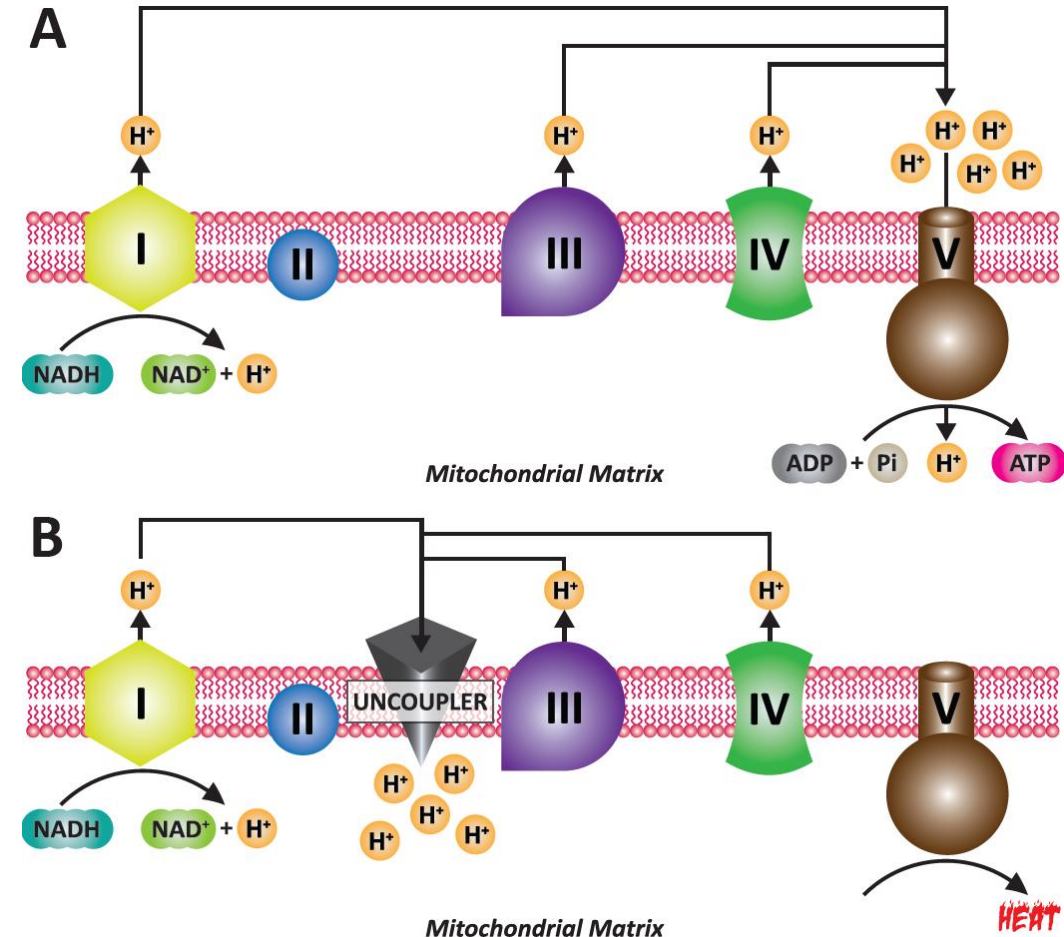
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How Thermogens Produce a Fever

Uncoupling of Oxidative Phosphorylation (UOP)

1. **Thermogenic** substance enters the body
2. Causes protons to leak across the mitochondrial membrane resulting in reduction of proton gradient
3. **Adenosine diphosphate (ADP)** is not phosphorylated to **adenosine triphosphate (ATP)**
4. The energy that would normally be used to produce **ATP** is instead released as heat
5. Heat is produced but process may be dose dependent



Source: Figure adapted from Song and Villeneuve (2021). Visualization of the uncoupling of oxidative phosphorylation: In "A," the proton gradient is maintained, and ATP is produced; while in "B," the uncoupler allows protons to leak across the membrane without generating ATP, resulting in reduced ATP production and the release of energy as heat.

How Pyrogens Produce a Fever

Alteration of the Thermoregulatory Center of the Brain

1. **Pyrogenic** substance enters the body
2. Causes host cells (primarily macrophages) to **release cytokines**, like IL-1 and IL-6
3. Cytokines travel to hypothalamic **thermoregulatory** centers to induce synthesis of prostaglandins
4. **Prostaglandins** alter the thermoregulatory center of the brain
5. **Fever** is established

Source: Sahib El-Radhi, A. (2019).



Pyrogens

- **Types:** Endotoxin and non-endotoxin pyrogens
- **Sources:** Endogenous (internal) and exogenous (external)
- **Fever Mechanism:** Cytokine upregulation

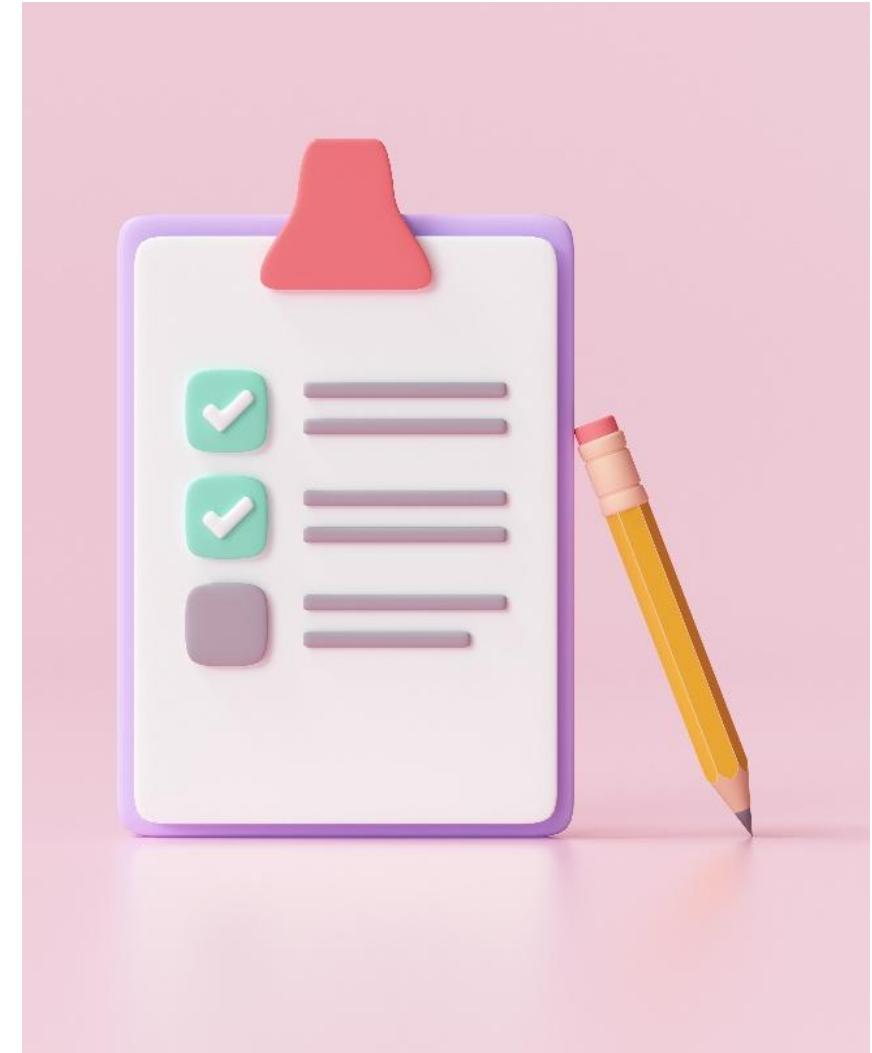
Type	Characteristics
Bacterial endotoxin	Lipopolysaccharides (LPS) derived from the cell walls of gram-negative bacteria; characteristically very resistant to heat
Non-endotoxin, endogenous	Cytokine signaling molecules (<i>e.g.</i> , IL-1, IL-6, TNF- α , IFN- γ)
Non-endotoxin, exogenous	Gram-positive bacteria (<i>e.g.</i> , peptidoglycan, lipoteichoic acids, and bacterial lipoproteins), fungi, yeast, viruses; biological sources that retain their activity

Types of Pyrogen Tests

Pyrogen Test	Pyrogen Detection	Details
Rabbit pyrogen test (RPT)	Endotoxins & Non-Endotoxins	Only approved method for MMP detection in/on medical devices; In vivo
Monocyte activation test (MAT)	Endotoxins & Non-Endotoxins	Well-established method for cytokine-induced pyrogenicity in pharmaceuticals; Uses human blood-derived monocytes to detect pyrogens; up to 100x more sensitive than the RPT but is more complex to perform
Bacterial endotoxin test (BET) (also called limulus amebocyte lysate [LAL] test)	Endotoxin only	Based on the clotting reaction of blue hemolymph extracts from horseshoe crab blood to detect endotoxins from gram-negative bacteria
Recombinant horseshoe crab factor C (rFC) assay	Endotoxin only	Factor C is activated by endotoxins to produce a measurable fluorescent end product

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Research Questions...

- 1 What evidence supports the "MMPs" in ISO 10993-11, Annex G?
- 2 Are there "MMPs" missing from ISO 10993-11, Annex G?
- 3 How often do "MMPs" appear in chemical characterization results?
- 4 What is the failure rate of the RPT?



Select Literature Search Results: Annex G Pyrogens

ISO 10993-11, Annex G Pyrogen	Substance Description	Evidence of Fever Activity? (2026)
Metals, such as nickel salts	Metallics	We say: No because ...

- Metals, such as nickel salts, cause fevers through two mechanisms:
 1. “Metal-fume fever.” **Inhaled welding fumes** (<1 µm) reach the alveoli, where metal oxides generate reactive oxygen species that **trigger pro-inflammatory cytokines** (Greenberg and Vearrier, 2015; Szűcs-Somlyó *et al.*, 2023a)
 2. Macrophage ingestion of **implant wear debris** drives cytokine upregulation (Purdue *et al.*, 2006; Hallab, 2009; Connors *et al.*, 2022)
- Both routes are driven by **particulates, not metallic materials of construction**; fever is **not** an adverse response typically associated with device wear debris

Select Literature Search Results: Annex G Pyrogens

ISO 10993-11, Annex G Pyrogen	Substance Description	Evidence of Fever Activity? (2026)
IL-1, IL-6, TNF- α , IFN- γ , Prostaglandin E2, Noradrenaline	Endogenous signaling molecules	Yes, pyrogenic
Serotonin		No
Polyadenylic acid, Polyuridylic acid, Polyriboinosinic acid, Polyribocytidylic acid	Synthetic double-stranded RNA molecules: "Inducers"	Yes, pyrogenic

- Fevers mediated through cytokine pathway; substances are **biological pyrogens**.

Select Literature Search Results: Annex G Pyrogens

ISO 10993-11, Annex G Pyrogen	Substance Description	Evidence of Fever Activity? (2026)
2,4-Dinitrophenol (2,4-DNP), 4,6-Dinitro-o-cresol	Uncouplers of Oxidative Phosphorylation (UOP)	Yes, Thermogenic
Picric acid (2,4,6-trinitrophenol)	Pseudo-uncoupler	No

- Heat produced through UOP; substances are **chemical thermogens**.

Literature Search Results: Other UOP Substances

- Notably Borton and Coleman (2026) identified **42 additional UOPs** ...
- UOP solubility in water and saline was investigated
- **Saline** is the **only extract** used in the RPT

Solution	Insoluble	Soluble
Saline	41 (91%)	4 (9%)

- Therefore, the **RPT cannot** detect **UOPs**

Literature Search Results: Key Points

The mechanism of action matters when it comes to an ISO 10993-1 Pyrogen Risk Assessment...

Thermogens

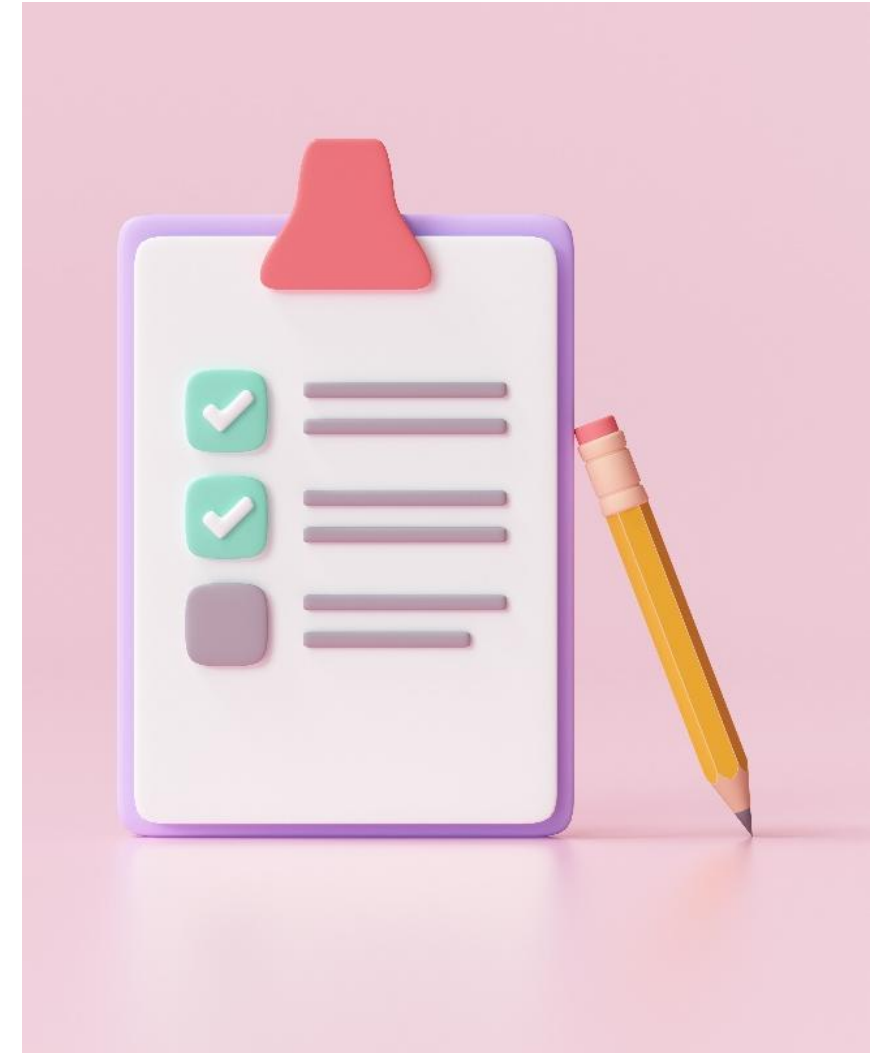
- UOPs cause a thermogenic response
- The RPT **cannot** detect UOPs that are **not** soluble in saline
 - Control through hazard assessment of materials of construction and manufacturing processes; chemical characterization + toxicological risk assessment

Biological Pyrogens

- Cytokine-mediated fevers
- Biologically-derived materials that maintain their biological characteristics (active cytokines, RNA, host proteins, etc.,)
 - Control through medical device manufacturing processes, routine monitoring, and sterilization

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RPT Data Mining Methods

- 5 contract research organizations surveyed
 - 7,167 RPTs reported
- Root cause data provided by 9 device manufacturers
 - Root causes reported based on 10 failures / 1,289 RPTs

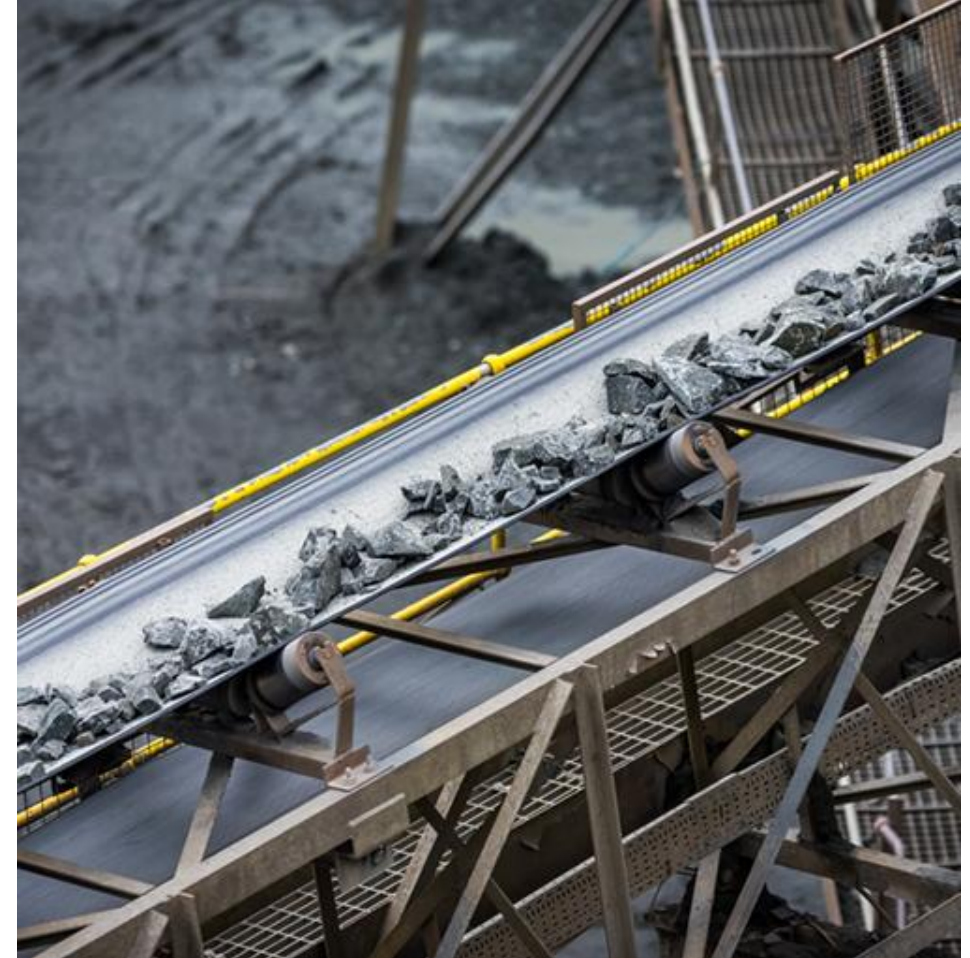


RPT Data Mining: CRO and Manufacturer Surveys

- Contract research organizations:
 - 7,167 RPTs reported, with a 2.65% average failure rate
 - Most failures are **not** repeatable: 0.31% fail after continuation testing
- Device manufacturers:
 - 9 manufacturers reported 1,289 RPTs with a 2.5% failure rate
 - Failures trace to **confounding variables**: endotoxin contamination or incompatibility with the RPT test system
- **Key Takeaway:**
 - 5 years of RPT data did **not** identify a single instance of a true "MMP"

Extractables Data Mining Methods

- CAS Nos. identified for Annex G "MMPs" and UOPs
 - Chemical names and CAS Nos. collected from labs, manufacturers, and consultants
- 12 firms surveyed across Europe and North America
 - ~18,000 chemical substances collated



Extractables Data Mining: Annex G CAS Nos. Survey

How often are Annex G substances and UOPs detected in medical device extracts?

Not often. . .

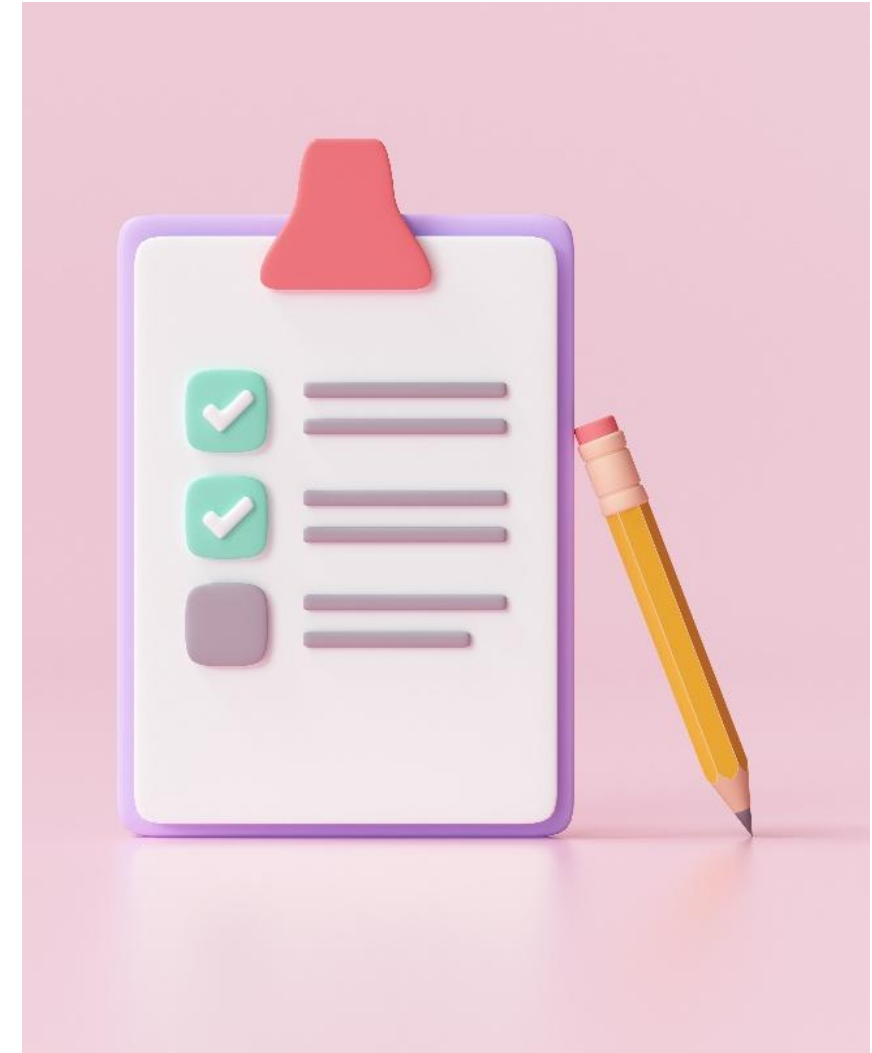
- Only **two** Annex G substances identified and **neither** produces fever in humans
- Only **three** UOPs identified, and **fever is not** the primary toxicity concern.

UOPs do **not** pose a **fever risk** to patients.

Annex G substances do **not** represent real pyrogen risk; **abandon the Annex G list** in favor of a real risk-based approach.

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Main Points



"MMPs" are either **biological pyrogens** or **chemical thermogens**. The mechanism of action is key to the pyrogen risk assessment.



The **RPT is inadequate** and should be retired.



Biological pyrogens: control via manufacturing processes or routine **MAT**.



Chemical thermogens are extremely rare and pose **no** patient risk.

Discussion and Questions



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