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

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**Background and Purpose:** Pyrogenicity presents a challenge to clinicians, medical device manufacturers, and regulators. A febrile response may be caused by endotoxin or non-endotoxin contamination or chemical agents that generate a material-mediated pyrogen (MMP) reaction. While test methods for the assessment of biological contamination are well established, MMP testing for medical devices remains controversial. The *in vivo* rabbit pyrogen test (RPT) is the "gold standard" for medical device pyrogenicity testing even though it has never been validated and its results are not directly transferable to humans. Annex G of ISO 10993-11:2017 lists 24 substances that are alleged to be medical device MMPs. This list includes cytokines, prostaglandins, neurotransmitters, inducers, drugs, uncouplers of oxidative phosphorylation (UOPs), naphthylamines, bacterial exotoxins, and metals. These compounds were further categorized by their origin as biological, chemical, drug, or uncoupler substances. The purpose of this project was to determine: (1) if the ISO Annex G MMPs are real; (2) whether they have been extracted from medical devices; (3) if any MMPs have been linked to RPT failures; and (4) whether alternative methods of evaluation would be acceptable.

**Methods:** To address these issues, a comprehensive data-mining effort was conducted for extracted chemicals and animal-testing results. In addition, a broad literature search was completed to identify MMPs associated with medical devices. First, chemical names and Chemical Abstracts Service (CAS) numbers were identified for the Annex G MMPs. Next, lists of chemicals extracted from medical devices were collected from device manufacturers, consulting companies, and analytical chemistry laboratories at contract research organizations (CROs). If a list of extractables was not supplied, the company provided the results of an internal search of its mass-spectral libraries for the MMP CAS numbers. A total of 13 firms contributed data. The Annex G CAS numbers and chemical names were subsequently screened against the collated list of 9,745 medical device extractables. Rabbit pyrogen testing data from CROs and medical device manufacturers was collected. Five CROs and eight medical device manufacturers furnished five years of RPT data (2019-2023), including the number of tests conducted annually and the number of failures. Where possible, root causes of the failures were provided by the device manufacturers. Pyrogenicity terms, MMP names, chemical names, and CAS numbers were among the keywords used in the literature search. The following sources were consulted: Embase, Scopus, PubMed, PubChem, ToxPlanet, and Perplexity AI.

**Results:** The literature search confirmed that Annex G's biological compounds (cytokines, prostaglandins, neurotransmitters, inducers, and exotoxins) plus Schedule I & II drugs are pyrogens; however, there was no evidence that naphthylamines were pyrogenic. Two of the three UOPs, 2,4-dinitrophenol and 4,6-dinitro-o-cresol, were determined to be human thermogens. The literature search located 44 additional UOPs, which were endogenous substances, pharmaceuticals, research compounds, pesticides/herbicides, or highly toxic chemicals that have been banned from human consumption or use. Overall, there were 47 UOPs of which 32 (68%) were drugs or endogenous substances. The UOPs were categorized as classical uncouplers and pseudo-uncouplers. Classical uncouplers produce a thermogenic response on their own, while pseudo-uncouplers only produce a fever response with assistance from other chemicals. One classical uncoupler, pentachlorophenol, which was identified in the literature search was subsequently found on the collated list of extractables from medical devices. In addition, seven long-chain fatty acids (C16-C20) were also identified in the

scientific literature and listed as medical device extractables. All are nutrients, four are endogenous, three are essential, none are pyrogens, while three are anti-pyrogenic. One is a classical uncoupler and four are pseudo-uncouplers. All are insoluble in water and saline. Of the Annex G MMPs, only 1-naphthylamine, which is non-pyrogenic, also appears on the medical device extractables list. Five CROs reported results from 7,167 RPT studies. The RPT failure rate ranged from 0.53% to 5.62%, with an average rate of 2.49% (or 1.71% when the outlier lab is excluded). Two labs reported failure rates from continuation studies performed following an initial failure. Only 0.84% of continuation studies failed. The eight medical device manufacturers reported a total of 1,289 RPT studies, with an initial failure rate of 2.5%. When root causes could be identified, the true failure rate fell to 0.40%.

**Conclusions:** The literature review confirmed the following about Annex G MMPs: (1) endogenous biological compounds and drugs produce a fever response in humans; (2) naphthylamines are not pyrogenic substances; (3) metals are only associated with fever under specific industrial inhalation exposure conditions; and (4) UOP substances, like 2,4-dinitrophenol and 4,6-dinitro-o-cresol, are not pyrogenic, instead they produce a thermogenic response that is not mediated through the cytokine network. The data-mining results showed that only one of the 24 Annex G MMPs had been extracted from medical devices and it was not a pyrogen. Further, data mining determined that the RPT failure rate was low; and that failures which were investigated were found to be caused by endotoxin lipopolysaccharide or incompatibilities with the RPT assay (*i.e.*, intravenous injection of viscous solutions) not any of the ISO MMPs. Uncouplers of oxidative phosphorylation are insoluble in saline, the sole extraction solvent used for the RPT. Consequently, even if UOPs were present in medical devices, it is very improbable that they would be exacted in saline and injected into rabbits. Thus, as there is little chance of UOPs leaching from medical devices, they do not present a pyrogenicity risk to patients. If UOPs appear in medical device extracts from chemical characterization studies, an ISO 10993-17:2023 toxicological risk assessment could determine if they present a health hazard to patients. Specifically, adverse effects of UOPs can be observed in systemic toxicity studies by the following benchmarks: increased body temperature/ hyperthermia, increased metabolic rate, increased respiration rate, increased heart rate, decreased body weight, and hepatotoxicity due to mitochondrial disfunction. Based on the low RPT failure rates and the fact that none of the pyrogenic Annex G substances were detected in medical device extracts, the prevalence of MMPs in medical devices is negligible. Hence, screening for MMPs in medical devices is not necessary. Annex G is fatally flawed because it contains physiological pyrogens, drugs, and chemicals that are highly unlikely to be in medical devices. This belief was confirmed by the data mining's CAS number search. Since it was determined that none of the Annex G pyrogens were identified in medical device extracts, ISO Technical Committee 194 should remove Annex G from the ISO 10993-11 standard. The primary reason the RPT continues to be used by the medical device industry is that it can detect UOPs. However, given that UOPs are not soluble in saline, it is extremely doubtful that they could be extracted from medical devices and injected into rabbits. Since it is not needed to detect MMPs, the RPT should be replaced by the *in vitro* monocyte activation test (MAT) that can detect all classes of pyrogens, including endotoxins and non-endotoxins, as well as yeasts, molds, and viruses. In recent years, the MAT, which was developed in 1996, has become the new global standard for pyrogen testing.