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The following comments are made in response to: FR Notice (Vol. 71, No. 238, pp. 74533-74534, 12/12/06), Scientific Peer Review Meeting on the Use of In Vitro Pyrogenicity Testing Methods; Request for Comments.

We would like to acknowledge the efforts that NICEATM and ICCVAM have made towards implementing *in vitro* testing as a replacement for that of the standard *in vivo* methods for pyrogenicity. Towards this common goal we are all in agreement. However, even though we share the goal of replacement of methods, which use animals wherever possible, we in the medical device industry have had to continue to use the rabbit pyrogen test to assure that new material components for our products do not contain substances known as "material-mediated" pyrogens. The known substances of this type, listed in ISP 10993-11 Annex F, are generally chemicals, which are mostly understood to directly stimulate the thermoregulatory center in the brain to produce a pyrogenic response. This type of non-endotoxin pyrogen is rare, I have been working in the medical device industry now since 2004 and in this capacity have never observed a pyrogen test conducted on a medical device that did induce a febrile reaction in an animal. This testing is performed by government mandate: Code of Federal Regulations, Title 21, (21CFR610.13) and as such, is not an option for the medical device industry. Further, ISO 10993-11:2006 Annex F states that medical devices containing new chemical entities or substances which have previously elicited a pyrogenic response, should be evaluated for material-mediated pyrogenicity.

The proposed *in vitro* methods for assessing pyrogenicity do not include any data that would support the validity of these methods for the indication of material-mediated pyrogenicity. *In vitro* pyrogen tests appear from studies cited and summarized to be a suitable substitute for the LAL test for endotoxin testing (which we use routinely for product lot release) with additional capability to detect pyrogenic substances from gram positive cell walls and fungi; but it is mechanistically unlikely these methods can detect the majority of materal-mediated pyrogens (Annex F list), because there is no macrophage/ cytokine involvement. To accept any/all of these methods as replacements

for the rabbit pyrogen test in all cases without data to support their intended use/s for the acceptance of medical devices would at the very least be deemed to be an equivocal representation for safety considerations in human practices. A minimum consideration should be given to a further study to evaluate some of the non-endotoxin material-mediated pyrogens contained in Annex F of the ISO 10993-11 document by the *in-vitro* pyrogen methods. We strongly recommend that such a study be initiated.

The ICCVAM background document itself notes the following items of concern regarding the assays:

- One identified limitation of the *in vitro* methods is the lack of data to determine their responses to, and suitability for, non-endotoxin pyrogens that are known to be detected by the RPT.
- ECVAM validation studies focused specifically on Gram-negative endotoxin due to the unavailability of standardized, non-endotoxin pyrogens
- ***In vitro* pyrogenicity test method validation studies should evaluate an adequate sample of substances and products of the types that are intended to be tested with these methods. The list of test substances selected for inclusion in the ECVAM validation studies consists solely of marketed parenteral pharmaceuticals that have been labeled as free from detectable pyrogens. No specific rationale was provided for the selection of these test substances.**
- A recognized limitation of the *in vitro* methods is the lack of data to determine their responses to, and suitability for, non-endotoxin pyrogens that are known to be detected by the RPT.

Further testing should be conducted using a representative sample of the types of material-mediated pyrogens as are found in Annex F of ISO 10993-11:2006. When testing of this nature is completed, then the data generated would be better suited for justification of the assays acceptance in the medical device industry. Until such testing is completed and data becomes available, it would be extremely difficult to justify the use of these assays for medical devices.

Respectfully submitted by:

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