April 13-14, 2011 EPA Human Studies Review Board Meeting Report

Appendix 1

Reconsideration of Two Concerns Previously Raised by the HSRB in its June 2009 Review of a Pre-Rule Intentional Human Dosing Study Involving Chlorpyrifos (Kisicki *et al.* 1999)

In addition to the scientific and ethics reviews of several completed studies and scenarios involving intentional exposure of human subjects to pesticides or other EPA regulated compounds requested by the Agency, at its April 2011 meeting the Board considered additional information regarding the Kisicki *et al*. (1999) study on chlorpyrifos, which was originally discussed by the HSRB in June 2009 (EPA HSRB 2009). Additional information was supplied by Dow AgroSciences about two concerns raised by the HSRB at the June 2009 meeting, but this information was not supplied to the HSRB until after the report of that meeting had been finalized.

In its June 2009 report, the Board raised four concerns about the Kisicki *et al.* (1999) study. Two of these concerns were addressed in the additional information provided by Dow AgroSciences.

The first of these concerns was about the substantially lower level of oral absorption of chlorpyrifos calculated in the Kisicki *et al.* (1999) study as compared with the level of absorption calculated in an earlier study conducted Nolan *et al*. (1982). The Kisicki *et al.* study reported 35% absorption as compared with the 70% absorption reported by Nolan *et al*. for the same dosing level (0.5 mg/kg). The Board was skeptical as to whether the reason presented for the differences between the level of oral absorption between the two studies, namely that the gelatin capsule used in the Kisicki *et al.* study would have taken longer to dissolve and would therefore have resulted in lower absorption, were valid.

The second of these concerns was the lack of documentation that urine samples were subjected to acid hydrolysis. Acid hydrolysis would be necessary to liberate the chlorpyrifos metabolite trichloropyridinol (TCP) from any conjugates in the sample. Had acid hydrolysis not been performed, the subsequent quantitation of TCP in the urine samples would have underestimated the levels of TCP. In its June 2009 report (EPA HSRB 2009) the Board concluded that, had hydrolysis step had not been performed, the lower oral absorption reported in the Kisicki *et al.* (1999) might have been the result of inaccurate measurement of urinary TCP levels.

The information provided to the Agency by Dow AgroSciences addressed both of these concerns. This supplementary information indicated that the urine was indeed subjected to acid hydrolysis using methods that had been previously shown to free TCP from any conjugates. The information provided by Dow AgroSciences also indicated that the chlorpyrifos used in the Kisicki *et al.* (1999) study was placed in a gelatin capsule in a crystalline form, with the remainder of the capsule filled with lactose. By contrast, in the Nolan *et al.* (1982) study the chlorpyrifos was dissolved in methylene chloride and applied as a solution to the lactose tablets. The particle size of the chlorpyrifos used in the Kisicki *et al.* study thus was larger than that used

in the Nolan *et al.* study. It is possible that these larger particles could have been absorbed more slowly than the smaller particles.

After considering this addition information, the Board concluded that its original recommendations about the Kisicki *et al.* (1999) study should be amended as follows:

1. It is logical that larger particles of a material such as chlorpyrifos would be absorbed more slowly than smaller particles. The differences in absorption between the Nolan *et al.* (1982) and Kisicki *et al.* (1999) studies may have resulted, at least in part, from the different sizes of chlorpyrifos particles in the two formulations.

2. The quantitation of urinary TCP was accurate because the urine was subjected to acid hydrolysis and heat to liberate conjugated TCP.

The Board also concluded, however, that other issues discussed by the HSRB at the June 2009 meeting remain unaddressed. These unaddressed and significant issues still raise concern about the reliability and utility of the blood and urine measurements of chlorpyrifos and/or TCP from Kisicki *et al.* (1999) for risk assessment purposes.

REFERENCES

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