Centre for Integrated Research in Biosafety

Tel: +64 3 364 2500, Fax: + 64 364 2590
Email: jack.heinemann@canterbury.ac.nz

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

18 July 2016

Comments on dossiers proposing harmonised classification and labelling of substances

Glyphosate and commercial formulations containing glyphosate

Having reviewed the risk assessment guidelines, we were unable to find evidence that the European Chemicals Agency considered the sub-lethal effects of microbial exposures to glyphosate-based herbicide formulations.

Without considering these effects, the ECHA **hazard identification** process is incomplete. As a fundamental first step in both an environmental and human health risk assessment, failure to evaluate a hazard affects all other aspects of the **risk assessment** and possible **risk management** options.

Herbicide with glyphosate induces a multiple antibiotic resistance (MAR) response in bacteria [1]. The MAR induces significant changes in the minimum inhibitory concentrations (MICs) of at least the antibiotics ciprofloxacin and kanamycin.

The effect was tested and found in two potential livestock and human pathogens, *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. It is thus likely to occur in other bacteria.

Up to 3-fold (300%) changes were observed in the MIC depending on species and antibiotic. These levels of change can be clinically relevant. For example, one of the last options for some *Staphylococcus aureus* infections is the antibiotic vancomycin [2]. Patients contracting *S. aureus* infections clinically considered treatable with vancomycin had significantly different outcomes depending on whether the infecting population had a vancomycin MIC of 1.5μg/ml or ≤1μg/ml [3]. The former were 2.4 times more likely to die or suffer a significant complication. This was true despite maintaining *in vivo* vancomycin levels at a minimum of 15μg/ml and no strain having an MIC >2μg/ml. Similarly, enteric fevers caused by *Salmonella typhi* and *Salmonella paratyphi* with decreased susceptibility to ciprofloxacin (MIC <1μg/ml) had significantly different clinical outcomes from those caused by ciprofloxacin-susceptible strains (MIC <0.125μg/ml) [4].

A study of hospitalised patients given the antibiotic ciprofloxacin found that a majority failed to reach efficacious doses for *E. coli, Klebsiella* spp., *Enterobacteriaceae, Proteus* spp. and *Pseudomonas aeruginosa* infections. In each case, the infection involved a ciprofloxacin-susceptible strain. According to the United States Food and Drug Administration, any strain with an MIC <1μg/ml is

considered susceptible to ciprofloxacin. A 2-fold change in the MIC of infecting strains, from ≤0.125μg/ml to ≤0.25μg/ml, was enough to cause 21% of patients to get a lower than target dose of the antibiotic. And when the MIC reached a 4-fold increase to ≤0.5μg/ml, 75% of patients failed to receive the target dose [5].

Moreover, glyphosate formulations at exposure concentrations lower than needed to induce a MAR response could, when combined with exposure to other chemicals, such as acetylsalicylic acid (aspirin), still induce the effect [1]. Thus, it would be inadequate to consider the effects of the herbicide, or herbicide exposures, alone. Instead, the ECHA should consider scenarios where herbicide, infection by bacterial pathogens, and other exposures to salicylic acid-based pain relief or other products might combine.

The MAR effect occurred at concentrations that were higher than currently allowed maximum residue limits in food. However, they were far below the application concentration and potential exposure concentrations from spray drift or feed that is not monitored for herbicide residues. Microbes in or on humans and animals may be exposed simultaneously with the human or animal, or may be exposed separately and transfer. Wildlife (particularly bees), livestock, farm workers, and rural families can be exposed to either or both inducing concentrations of the herbicide or bacteria that have been induced and then taken up through food or by inhalation. Indeed, for example, downwind of concentrated animal feeding operations (CAFOs) there is a significant wind carriage of fecal microbes [6]. In some countries beehives are treated with antibiotics. The hives, or the bee flora itself, may receive inducing exposures during spraying thus compromising antibiotic efficacy.

The **potential adverse effects** of raising the MICs of antibiotics include higher therapy failure rates (medicine, veterinary medicine), longer infectious periods with higher likelihood of sequela (medicine, veterinary medicine, treatment of beehives) and, importantly, a demand for higher antibiotic use to retain efficacy (animal feed, medicine and veterinary medicine).

We therefore recommend that the ECHA formally considers the sub-lethal effects of herbicide exposures on bacteria in both the human health and environmental risk assessments. The assessment should be based on the relevant commercial formulations to take into account all ingredients – active and inert - that might induce a change in bacteria responses to antibiotics.

Submitted by

Professor Jack A. Heinemann, PhD
Centre for Integrated Research in Biosafety
University of Canterbury
Christchurch, New Zealand

1 Kurenbach, B., Marjoshi, D., Amabile-Cuevas, C., Ferguson, G. C., Godsoe, W., Gibson, P. & Heinemann, J. A. Sub-lethal exposure to commercial formulations of the herbicides dicamba, 2,4-D and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella

enterica serovar Typhimurium. *mBIO* **6**, e00009-00015, doi:10.1128/mBio.00009-15 (2015).

2 CDC. Antibiotic resistance threats in the United States, 2013. (Centers for Disease Control and Prevention, 2014).

3 Lodise, T. P., Graves, J., Evans, A., Graffunder, E., Helmecke, M., Lomaestro, B. M. & Stellrecht, K. Relationship between vancomycin MIC and failure among patients with methicillin-resistant Staphylococcus aureus bacteremia treated with vancomycin. *Antimicrob. Agents. Chemother.* **52**, 3315-2230 (2008).

4 Hassing, R. J., Goessens, W. H. F., Mevius, D. J., Pelt, W., Mouton, J. W., Verbon, A. & Genderen, P. J. Decreased ciprofloxacin susceptibility in Salmonella Typhi and Paratyphi infections in ill-returned travellers: the impact on clinical outcome and future treatment options. *Eu. J. Clin. Microbiol. Infect. Dis.* **32**, 1295-1301, doi:10.1007/s10096-013-1878-9 (2013).

5 Haeseker, M., Stolk, L., Nieman, F., Hoebe, C., Neef, C., Bruggeman, C. & Verbon, A. The ciprofloxacin target AUC : MIC ratio is not reached in hospitalized patients with the recommended dosing regimens. *Br. J. Clin. Pharm.* **75**, 180-185, doi:10.1111/j.1365-2125.2012.04337.x (2013).

6 McEachran, A. D., Blackwell, B. R., Hanson, J. D., Wooten, K. J., Mayer, G. D., Cox, S. B. & Smith, P. N. Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. *Environ. Health Pers.* **123**, 337-343, doi:10.1289/ehp.1408555 (2015).