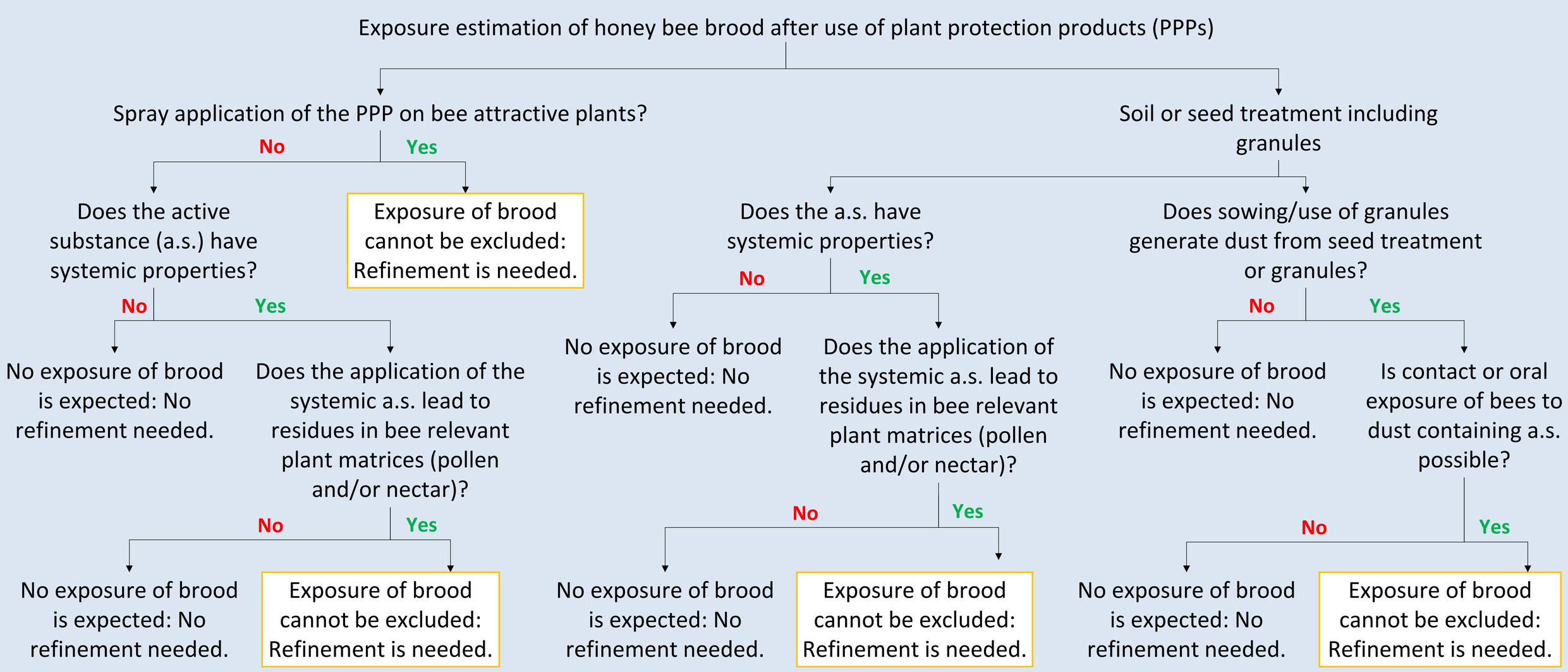
Conceptual framework for the selection of higher-tier refinement options with focus on honey bee (*Apis mellifera*) brood

on behalf of the International Commission for Plant-Pollinator Relationships (ICPPR) Bee Brood Working Group

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Higher-tier refinement options with focus on honey bee brood (*Apis mellifera* L.)



Study type (randomized order)	Advantages	Limitations
OECD 75 GD and/or EPPO 170 (semi-field)	• Evaluates actual application conditions • Integration of multiple exposure routes • Controlled, worst-case exposure scenario in a highly bee attractive crop • When considering fungicides or insecticides, allows assessment of pre-flower applications • Ring-tested design (2007 with fenoxycarb) • Can be combined or seen as an extension of the EPPO 170 (semi-field)	• Application of a herbicide invariably creates an adverse impact on forage crop in the tunnel, leading to reduced exposure time • High variability in brood termination rate between colonies of the same treatment group can occur • Enclosure stress on colony under semi-field (tunnel) conditions may cause reduction in the number of bee brood (*i.e.*, caging effect) • Crops not attractive to bees are less suitable • Exposure/foraging period is limited to flowering period (*i.e.*, 7-10 days) • Test can be impacted by adverse climatic conditions
Colony Feeding Study (Oomen *et al*., 1992)	• Acute (single) feeding ensures exposure of adult bees and brood • Less resource- and space-intensive • Feeding ensures continued exposure • Screening of potential effects at the colony level • Design facilitates testing of herbicides • Low variability of brood termination rate • Relatively independent of weather conditions	• High exposure scenario (concentration reflects recommended field use) • Partially controlled exposure of brood: Forager bees will collect nectar and pollen from other sources • Reduced palatability of the feeding solution and variable exposure as food may simply be stored in combs rather than consumed
Colony Feeding Study (Lückmann & Schmitzer, 2019)	• See Study Type "Colony Feeding Study (Oomen *et al*., 1992)" • Chronic feeding exposure of adult bees and brood • Could provide a more realistic exposure scenario if test concentration is based on chronic larval or adult endpoints or considering residue exposure ratios • Link to residue studies (if available) • Ring-tested design (2013 with fenoxycarb)	• See Study Type "Colony Feeding Study (Oomen *et al*., 1992)" • Requires pre-information gained from residue studies on residue levels or endpoints from chronic larval and adult studies
Large Colony Feeding Study	• See Study Type "Colony Feeding Study (Lückmann & Schmitzer, 2019)" • Colony-level dose response relationship • Allows for long time whole-colony developmental assessments (covering overwintering) associated with the long term exposure scenario	• See Study Type "Colony Feeding Study (Lückmann & Schmitzer, 2019)" • Resource- and time-intensive
EPPO 170 (field) and EPA 850.3040	• Field realistic exposure scenario • Evaluates actual application conditions • When considering fungicides or insecticides, allows assessment of pre-flower applications • Can be extended by brood assessments according to OECD 75 GD • Low variability of brood termination rate	• Generally, the number of replicates is low • Crops not attractive to bees are less suitable • Application of a herbicide invariably creates an adverse impact on forage crop in the tunnel, leading to reduced exposure time • The test can be impacted by adverse climatic conditions
Residue Study	• Linking field realistic residues (*e.g.*, pollen and/or nectar) with endpoints from laboratory studies • Possible consideration/inclusion of bees for collection of nectar and pollen	• Colonies used for residue collection cannot be used for biological assessments (*i.e.*, brood assessments) • Test can be impacted by adverse climatic conditions