IN THE CIRCUIT COURT
TWENTIETH JUDICIAL CIRCUIT
ST. CLAIR COUNTY, ILLINOIS

DIANA HOFFMANN, et al., Plaintiffs, v. SYNGENTA CROP PROTECTION, LLC, SYNGENTA AG, CHEVRON U.S.A., INC., and GROWMARK INC., Defendants.	No. 17-L-517

CONFIDENTIAL

Rebuttal Expert Report
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For

Korein Tillery, LLC
505 North 7th Street, Suite 3600
St. Louis, MO 63101

By

Beate Ritz, M.D., Ph.D.

Shresta et al (2020) AHS study critique

The Shresta et al. (2020) study represents the latest instalment of the AHS study on the use of pesticides and Parkinson's Disease (PD) in the Agricultural Health Study (AHS). While the AHS cohort started with more than 89,000[1] participants at enrolment in the early to mid-1990s, this paper relies on only 38,274 pesticide applicators and 27,836 of their spouses actively followed, i.e., those who completed at least one additional follow-up survey after baseline. Of note, after additional exclusions due to incomplete data and inconsistent information on PD, the authors included 38,274 applicators and 27,836 spouses in their 'ever/never use' of pesticides analyses. For analyses on any more detail than ever/never use of pesticides - including paraquat use - the sample size was even smaller as only 19,068 male farmers out of the 52,394 originally enrolled reported details of their paraquat use in the baseline take home questionnaires and were actively followed over time (36.4% of those enrolled). Thus, this cohort suffered an enormous loss to active follow-up, especially among male enrollees. Generally, a cohort study is considered less subject to bias from loss to follow-up if it has a 90% or greater completion of follow-up by its participants (see also Greenland 1977). There are some examples of excellent response rates during active follow-up in cohorts- one being the Nurses' Health Study; specifically, three Nurses' Health Studies (starting in 1976, 1986, and 2010) had an overall active response rate for participants of 86.2% as of 2012 (with 35+ years of follow-up for the NHS I study at that time), and a 90% to 94% response rate for those who completed more than a year in the study for the NHS III - which started in 2010 (Bao et al. 2016). One major problem with the tremendous loss to active follow-up in the AHS cohort (36.1% of male farmers were lost at the first and 53.8% at the second follow-up survey) is the potential for an outcome other than cancer to be incorrectly recorded. Making a diagnoses of non-cancer diseases such as PD depends overwhelmingly on active participation during follow-up (note: this is not the case for cancers as these can be ascertained passively via cancer registry linkage and from mortality records for those that are fatal; PD is not often reported as a direct cause of death and is under-reported on death certificates). Another main issue for a loss to active follow-up in the AHS is the potential for exposure misclassification for all pesticide exposures that occurred after baseline. Active follow-up that includes surveying farmers is needed to update exposure

[1] https://aghealth.nih.gov/participants/ 52,394 (licensed private pesticide applicators) +32,345 (spouses) + 4,916 (commercial pesticide applicators) = 89,655 participants

histories over follow-up time since there is no pesticide use registry, i.e., farmers have to self-report their use. Thus, updating exposure information during follow-up depended on active participation of AHS cohort members. The potential to wrongly assign exposure status or the average amount/frequency of exposure for a study participant increases with increasing follow-up time during which pesticide use in active farmers changed but was not recorded due to lack of their response to a follow-up survey. Shresta et al.'s analyses presented here relied most heavily on baseline exposure data collected at enrolment (ever/never paraquat use) and on a take-home questionnaire that contained details on frequency and duration of paraquat use but was returned by only 44% of all farmers who were enrolled; in a subgroup analysis they also used pesticide data collected at the second survey between 1999 and 2004 at which time 36.1% of farmers did not respond anymore and were lost to follow-up. Therefore, the Shresta et al. analyses to a great extent ignored exposures that occurred after enrolment or after the second survey (1999-2004) which would be most problematic for PD cases diagnosed in later follow-up periods for whom exposures were unknown and thus not counted after 1999-2004, i.e., the first follow-up survey. For example the use of paraquat on soybeans in the US increased dramatically after 2004 (see: https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=1999&map=PARAQUAT&hilo=L) and soybeans are a major crop farmed by Iowa farmers who contributed 61% of all PD cases identified in the AHS for the Shresta et al. analyses. It is also important to note that most PD cases indeed accumulated in the AHS after 2004, i.e., 527 (88%) of all potential incident PD cases Shresta et al. relied on were identified after follow-up survey 2 and the FAME nested case-control study by Tanner et al. (Supplemental Figure 2). This may explain at least in part the observation that generally pesticide associations with PD were seen and stronger in the first 10 years of follow-up (see also below and Supplemental Table 3). In fact, for one of the pesticides the authors singled out as associated with PD, trifluralin, only the first 10 years showed an association (HR= 1.61; 95% CI 1.18, 2.20) while the HR dropped to 1.08 (95%CI 0.79, 1.47) after 10 years of follow-up and this difference in estimates for those with shorter versus longer follow-up was formally statistically significant.

Loss to follow-up: The AHS cohort study started enrollment at baseline in 1993 and was designed to follow farmer/commercial pesticide applicators in North Carolina and Iowa prospectively for cancer outcome through registries and for other health outcomes in subsequent interviews with cohort members. Applicators and farmers were recruited as they attended

pesticide-licensing exams in each state and spouses through take-home questionnaires the applicators took home. At the pesticide licensing exam, the AHS respondents were only asked to provide a yes/no answer to the question "having ever used paraquat" while details about their long-term pesticide use history was obtained via a take home questionnaire they had to mail back. Notably, while over 80% of licensed applicators (52,394 farmers) enrolled in the study by completing the on-site form, only 44% (22,916) returned the second questionnaire that elicited more detailed information about paraquat use. Importantly, the Phase 2 survey that first elicited outcome information on PD was completed by 33,456 applicators and 23,796 spouses; similarly, PD was assessed in Phase 3 for 24,170 applicators and 19,959 spouses, and in Phase 4 for 24, 145 applicators and 18,186 spouses; i.e., the cohort suffered a great loss to follow-up over the 20 years of follow-up (see above). Such a loss is a very important problem in a cohort study for a disease such as PD that cannot be identified from a disease registry or even death records as was done for the primary outcome (cancer) for which the AHS was designed. Specifically, for PD – a disease without a registry in Iowa and NC that is also chronically under-reported on death records - we would be extremely concerned about loss to follow-up of subjects who get a disease diagnosis and do not return to be surveyed. More importantly, this large loss also makes it more likely that such a loss was 'differential,' i.e., that pesticide exposed cases were lost at a higher rate than unexposed cases because farmers who suffered the most exposure may have been least likely to remain active cohort members and respond to further questions after they were diagnosed with PD. One reason for such differential loss may be the onset of depression in those most exposed to pesticides as pesticide exposure has been shown in the AHS (Beseler et al. 2008) and a French study (Weisskopf et al. 2013) to be associated with depression. Also, those diagnosed with PD have a 2-3 fold higher rate of suffering from depression prior to onset of PD (Shen et al. 2013; Jacob et al. 2010), and individuals who are depressed are less likely to be motivated to participate in a survey. Such a differential loss depending on higher exposure is in fact strongly suggested by results presented in Shresta's supplementary tables 1 and 3. Table 1 depicts PD risk according to life-time days of ever use of pesticides and - counter to any expectations from the general literature on pesticides and PD risk - male applicators had a reduced risk of developing (incident) PD with increasing days of ever use of pesticides with the highest number of days showing the strongest negative association (HR of 0.79; 95% CI 0.59-1.06). First, this negative trend is counterintuitive and would not be expected, i.e., under the null

hypothesis one would expect no association with PD risk with lifetime days of ever using pesticides if pesticides had no effect on PD. Alternatively, we would even expect a positive association under the assumption that the existing literature is correct, i.e., the consensus being that 'pesticides' influence PD risk in general. A negative trend with PD is likely the result of a selective drop-out of the heaviest pesticide exposed male farmers who developed PD. Interestingly, this paradoxical result is not seen in female spouses i.e. they indeed showed an increased risk of incident PD (HR of 1.58; 95% CI 1.00-2.5, formally statistically significant) at the highest number of lifetime days of pesticide use. This result that females in the AHS are more representative of the true or expected estimate for PD risk with lifetime days of ever pesticide use is not only in line with what we would expect given the literature, but also supported by two additional facts. First, females truly volunteered for the AHS when sending back questionnaires at baseline with an intention to remain active cohort members - different from the male farmers who may have felt obliged at a licensing exam to fill out the AHS questionnaire but had no intention to truly be active cohort members and to provide follow-up data (this assumption is strongly supported by the only 44% return of take-home questionnaires by male farmers and the high drop-out rate in males at the second survey). Second, females have a much lower overall drop-out rate over follow-up, and were more willing to be active cohort members (46% of the original phase I male enrollees and 56% of female enrollees were still active in phase 4). Finally, as shown in supplemental table 3, in male applicators who were followed for less than 10 years, paraquat exposure indeed showed a positive association with PD (HR 1.21; 95% CI 0.86-1.72), while for those followed for a longer time the estimated hazard ratio reduced to 1 (the null value). This same phenomenon holds true for all other herbicides in this male cohort, i.e., only for the men with the shorter follow-up were hazard ratios increased and then reduced to 1 (the null value) with longer follow-up. This was seen even for pesticides the authors highlight as being associated with PD. This observation strongly supports the notion that there was selective loss to follow-up of PD patients who were most exposed to pesticides. Finally, a paradoxical observation was also made for head injuries: the AHS reported an inverse association between having a head injury and incident PD with an HR of 0.71 (95% CI: 0.46, 1.09). Thus, in the AHS head injury seemed 'protective' against incident PD while the literature is full of studies reporting positive associations between PD and head injury. The strongest data on head injuries comes from a large study in Denmark where the risk of having a medically

documented head injury strongly increased in the year before PD diagnosis; the authors attributed this to motor dysfunction in prodromal PD that causes falls and head injuries (Rugbjerg et al. 2008). Thus, the finding that head injuries are inversely associated with PD in the AHS provides a strong argument for PD patients with head injuries to also have been selected out of the AHS, having been selectively lost to follow-up at a higher rate. The authors also examined pesticide exposures and PD risk according to reported lifetime days of use of specific pesticides for a subgroup who reported this data on take home questionnaires. In Table 4 they present an inverted U-shaped pattern of risk increase with paraquat exposure that again suggests a selective loss to follow-up of the most exposed subjects (the same inverted U-shape pattern with a lower risk at highest lifetime days of exposure was also seen for two of the three pesticides the authors reported as being associated with PD risk – 2,4,5 T and terbufos). While the AHS researchers conducted a sensitivity analysis for the 'potential impact of loss-to-follow up using inverse probability of censoring weights', this analysis rests on assumptions that may not have been met and it was also only conducted in a much smaller subgroup of male applicators with more detailed pesticide application data. Specifically, the authors conducted "weighted Cox models to estimate HRs and 95% CIs, adjusting for covariates and using stabilized inverse probability weights." The denominator of the weights was the "probability of overall participation in Phase 4 conditional on exposure, year and baseline covariates (age, sex, education, smoking, alcohol use, state of residence; missing values imputed for covariates whenever applicable)," and the numerator of the weights was "the probability of overall participation in Phase 4 conditional only on year." This approach assumes that the outcome in combination with exposures and possibly other co-morbidities such as depression does not influence the drop-out rate and also that exposure measured at enrolment and the first follow-up is an adequate measure of cumulative pesticide exposure which, at least for paraquat with increasing use after 2004 on soybeans, is an incorrect assumption.

Exposure misclassification. There are important problems with exposure assessment in the AHS that include the type of questions asked for paraquat at enrolment (only yes/no at the licensing exam and more details about use in the take home questionnaire that was returned by only 44% of all farmers) and extend to the flawed assessment of exposures at follow-up for those who did not return actively after Phase 1 for whom exposures then were imputed according to a model that makes some questionable assumptions as it did not consider health outcomes.

Sheppard and Schaffer (2019) previously questioned the validity of the imputation procedure used in the AHS to assign exposures to those who did not return for a follow-up survey, i.e., a procedure that did not take outcome into consideration; these authors concluded that "Unfortunately, it is unlikely that the conclusion of Heltshe et al. (4)—'This multiple imputation will allow for bias reduction and improved efficiency in future analyses of the AHS'— is correct." Most importantly, it is well recognized that recall errors may affect exposures measures that rely on self-reported past exposures. These errors are likely to contribute to 'non-differential' exposure misclassification if exposure reporting occurred prior to disease onset as would be the case for incident PD, i.e., the exposures assessed at enrolment in the AHS (note: this was also acknowledged as a limitation by Shresta et al.). This type of misclassification is also most likely to underestimate any true effects, known as a 'bias towards the null,' and we are likely to not find any association even if one truly exists. Such errors may occur, for example, among applicators who used many different pesticides, who might get more easily confused about which ones they used or when or for how long they used, than farmers who used pesticides occasionally or used only a few specific pesticides. Recent use might be less error prone or reported more accurately while use that happened a long time ago might have been forgotten; also, older or less educated participants might have worse recall or fewer records to consult than younger or more educated participants. This type of exposure misclassification is most likely to have occurred in the AHS as those who remain healthy and those who later develop a disease are similarly likely to make mistakes and misreport exposures, i.e., disease does not influence the exposure reporting as it is not known who will become diseased. Non-differential recall error can be reduced through improved data collection efforts such as can be conducted at home visits to farmers by trained researchers who interact with the subject in interviews or may review pesticide purchasing records as can easily be done in case control studies such as a French case control study of 800 farmers (Elbaz et al. 2009) and the Tanner et al. (2011) FAME case control study nested within the AHS. Contrary to this, a great disadvantage of a large cohort study, such as the AHS, is that such in-depth person-by-person exposure assessment is too burdensome and expensive to achieve; in fact, cohorts generally collect lower quality exposure data on a much larger number of study subjects, substituting quantity for quality of data and encouraging 'non-differential' exposure misclassification bias that most likely leads to underestimation of true effects. This is known as drowning a signal out by the noise in the data, and the greater the

noise, the stronger any true signal (effect) has to be in order to be noticed above the noise that is generated by measurement error. The AHS study is most likely affected by non-differential exposure measurement error while the FAME study nested within the AHS put considerable efforts during in-person interviews and home visits into exposure assessment. Shresta et al. in fact compared the number and percent of cases and controls who reported ever/never paraquat use at enrolment in the AHS with the FAME study exposure assessment, and from Supplemental Table 8 it is possible to calculate sensitivity and specificity for exposure measures. Using the FAME study as the 'gold' standard for pesticide exposure assessment, I calculated from this table that the baseline enrolment questionnaire had a rather low sensitivity (less than 70%).

The authors themselves acknowledge that their conclusions regarding paraquat are limited:

> Specifically, for the herbicide paraquat, animal and earlier human studies offer persuasive evidence for a potential link with PD, despite continuing debate (Goldman et al., 2017; Jones et al., 2014). Some subgroups, including those with specific genetic makeup, head injury, and certain dietary intake have been found particularly vulnerable to PD following paraquat exposure (Goldman et al., 2012; Kamel et al., 2014; Lee et al., 2012; Ritz et al., 2009). We cannot rule out the possibility that limited evidence of independent associations between PD and ever-use of some pesticides (including paraquat) in the current study resulted from non-differential bias attenuating HR estimates; for example, the HR for ever-use of paraquat was elevated [HR: 1.09 (95% CI: 0.84, 1.41)], but not statistically significant. Nevertheless, we were still able to observe associations among those potentially more susceptible due to head injury.

The Shrestha et al study does not change any of my opinions as previously stated in my July 10, 2020 report.

Beate Ritz, M.D., Ph.D.

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