ECHA RAC Review of (S)-metolachlor

Carcinogenicity Classification: Additional Epidemiology Data

Key Messages

- There is no clear link between S-metolachlor exposure and incidence of cancer in humans.
- Additional epidemiology data for S-metolachlor is available from the AGRICAN cohort, strengthening the absence of a link between S-metolachlor exposure and increased incidence of tumours (See appendix 1 for reference)
- Mode of action data conducted following the IPCS and ILSI/HESI framework concluded activation of CAR in human hepatocytes did not result in cell proliferation, a key event in the formation of tumours; therefore S-metolachlor is considered not to be carcinogenic in humans.
- The mouse carcinogenicity study (which unusually ran for 2 years) should be included in the classification weight of evidence assessment as survival was >80% at 18 months (as recommended by OECD guidance) and provides evidence for a lack of carcinogenicity potential of S-metolachlor in a second species.

Background

(S)-metolachlor has been assessed for its carcinogenic potential through two carcinogenicity studies in rodents (rat and mouse), a mode of action data package and an evaluation of the relevant epidemiology literature.

In the two year rat carcinogenicity study, metolachlor caused an increase in liver tumours at the top dose in female rats only. Apart from the findings in the liver, there is no increased incidence in any tumour type that is statistically significant or biologically relevant with respect to the concurrent control in the rat study.

Due to the incidence of liver tumours in the rat study, a mode of action (MoA) investigation was conducted by Syngenta according to the International Programme on Chemical Safety (IPCS) and International Life Science Institute (ILSI) framework. As part of this, the potential for all alternative MoA were excluded. This investigation demonstrated that the liver tumours were due to a CAR MoA and therefore non-relevant to humans.

In the two year mouse carcinogenicity study, no evidence of neoplastic findings were observed. However, the 2 year mouse carcinogenicity study was deemed unacceptable for inclusion in hazard assessment, on the basis of poor survival at a later time point than these studies would normally be run. Syngenta consider that this study provides useful information and the study should be used as part of the weight of evidence for a decision on classification.

A number of epidemiology literature papers investigate the link between exposure to (S)-metolachlor and cancer incidence.

Comments provided during the public consultation on the classification of (S)-metolachlor requested a detailed strength of the evidence discussion to establish the appropriate category for classification for carcinogenicity. To support the discussion Syngenta have requested an independent expert in epidemiology to review the available literature presented in the CLH report and to request the inclusion of the latest publications from the AGRICAN cohort (Leon et al 2019; Lerro et al 2018, 2019 and 2020) in the discussion on cancer classification.

This document has been prepared to include additional data made available after the public consultation and to provide some additional information for (S)-metolachlor.

Epidemiology and Evidence of Human Carcinogenicity

Syngenta have requested an independent expert in epidemiology to re-review the available literature presented in the CLH dossier, in particular Silver *et al* (2015), which was highlighted during the public commenting as potentially demonstrating a link between (S)-metolachlor exposure and incidence of liver tumours. Based on the literature presented in the CLH dossier, the epidemiology expert concluded that there is no clear epidemiological evidence that (S)-metolachlor is associated with cancer in humans and an increased incidence in liver tumours.

Since the CLH dossier was submitted, additional data have become available from the AGRICAN cohort (Leon et al 2019; Lerro et al 2018, 2019 and 2020), which is much larger than the AHS cohort analysed in the Silver *et al* paper. These data are also included in the expert epidemiologist's review. In the AGRICAN cohort, no link has been observed between (S)-metolachlor exposure and an increased incidence of tumours.

Appendix 1 presents the full position statement from the independent epidemiology expert on the available epidemiology literature presented in the CLH dossier and also the AGRICAN literature studies.

The overall conclusion from the independent epidemiologist is as follows:

"Silver et al (2015) reported some suggestive evidence that liver cancer and follicular cell lymphoma may be associated with metolachlor exposure. However, there were no statistically significant increases in risk for applicators in any quartile of exposure when the analysis was performed using the most appropriate reference group consisting of applicators with the lowest quartile of exposure, and no significant trends with exposure. Furthermore, even in analyses using applicants unexposed to metolachlor as the reference group, trends with exposure were much weaker for the intensity-weighted exposure metric and barely reached statistical significance. For follicular cell lymphoma, additional evidence is available from a combined analysis of the AHS cohort and the much larger French AGRICAN cohort which included similar numbers of users of metolachlor. The combined analysis reported no evidence of an association between follicular cell lymphoma and metolachlor exposure. There is also no supporting evidence of an association between liver cancer and metolachlor from the AHS or other epidemiological studies, and Silver et al (2015) noted that further follow-up is needed to facilitate assessment of whether the differences in the results reflect greater statistical power with a larger reference category or other exposure related factors. It is also noted that further follow-up would permit better assessment of the role of latency in these associations and whether a longer lag period may be more biologically plausible. There is no persuasive evidence that any other cancer subtype (including lung, colon and prostate) is associated with metolachlor exposure."

Based on the conclusion from the epidemiologist, there is no clear link between (S)-metolachlor exposure and an increased incidence of liver tumours. Therefore, the published epidemiology literature available for (S)-metolachlor does not demonstrate evidence of a carcinogenic hazard to humans according to the CLP criteria for 1B (H350) classification.

Human Hepatocyte Studies- Mode of Action Studies

A mode of action data package was conducted according to the International Programme on Chemical Safety (IPCS) and International Life Science Institute (ILSI) framework for (S)-metolachlor to demonstrate that the liver tumours observed in female rats only were non-relevant to humans. The mode of action studies demonstrated that the liver tumours observed in the rat were due to a CAR mode of action, which is non-relevant to humans. Additionally, all other alternative modes of action which could be human relevant were excluded according to the IPCS and ILSI frameworks.

While the mode of action data package has some deficiencies, the data from human hepatocytes supports the findings from the epidemiology that there is no clear evidence that (S)-metolachlor is associated with liver tumours in humans (Cowie and Green, 2019).

For cancer in laboratory animals or humans to occur, cells need to proliferate. This a pre-requisite for the clonal expansion that causes a tumour. Human hepatocytes, from multiple human donors, do not proliferate following treatment with (S)-metolachlor. This demonstrates that the cancer observed in rat liver is not relevant to man.

The use of three independent human hepatocyte donors is considered sufficient to assess human non-relevance.

Human hepatocyte experiments are conducted with appropriate positive controls to demonstrate and ensure the validity of the experimental test system. (S)-metolachlor concentrations used in human hepatocyte studies take into account the maximum concentration that did not induce significant reductions in cell viability, ensuring that human hazard is fully assessed.

Overall, the human hepatocyte data from the mode of action data package supports the weight of evidence and Syngenta's position that (S)-metolachlor is unlikely to be carcinogenic to humans.

Mouse Carcinogenicity Study

The dossier submitter considered the mouse carcinogenicity study to be "not acceptable" based on survival rates of less than 50% at 104 weeks.

Unusually for a mouse carcinogenicity study, the study with metolachlor was performed over 24 months (104 weeks) rather than 18 months or 80 weeks as recommended by the current OECD test guideline. This study was initiated prior to the introduction of OECD test guideline 453, technical guidance document 116 and US EPA OPPTS 870.4300 Combined Chronic Toxicity/Carcinogenicity guideline (1998).

According to OECD guidance document 116 (2014), for a negative result to be acceptable in a carcinogenicity study, survival should be no less than 50% at 18 months (paragraph 162). The table below shows that in the metolachlor mouse study survival in any treatment group was no less than 73% and the overall female mean was 81% at this time point.

Examination of the data from the mouse study to determine how many mice died or were sacrificed moribund prior to the Week 79 interim sacrifice is summarised in the table below. For the animals which died prior to Week 79 and between week 79 and week 104, no increased incidence in early neoplastic or non-neoplastic lesions were observed above the concurrent control group which could be attributed to metolachlor. Overall in the 104 week mouse study, there was no evidence of an increase in neoplastic findings at any dose level.

Groups	1M	2M	3M	4M	5F	6F	7F	8F
N*	70	70	70	70	70	70	70	70
Number dying prior to Week 79	10	9	7	9	7	15	12	19
% survival to Week 79	86	87	90	87	90	79	83	73
% survival per sex	88				81			

*excludes animals sacrificed after 52 weeks of treatment…

The mouse carcinogenicity study is therefore valid for the assessment of the carcinogenicity potential of metolachlor and while there are some technical deviations from the current OECD test guideline, it should be considered as part of the weight of evidence approach for classification.

Overall Summary

Based the additional weight of evidence placed on epidemiology data during public commenting, Syngenta have re-reviewed their current position relating to classification for carcinogenicity. This includes a re-review of the epidemiology literature by an independent expert epidemiologist.

Based on the positions presented in the public commenting:

- In the re-review of the epidemiology data by an independent expert, no clear evidence of carcinogenicity was observed in humans. This includes the incidence of liver tumours, which were only observed in the rat study. In this re-review, additional literature was reviewed from the AGRICAN cohort of studies which did not demonstrate an increased incidence of tumours in humans.
- Additionally, although there are some limitations on the mode of action data, the human hepatocyte studies clearly demonstrate that cell proliferation does not occur following exposure to (S)-metolachlor. Cell proliferation is a key initiating event to the formation of tumours.
- The mouse study is acceptable for inclusion in the hazard assessment and should be included as part

of the weight of evidence for classification. In this study metolachlor did not influence the frequency of neoplastic and non-neoplastic tumours after 104 weeks

S-metolachlor does not pose a carcinogenic hazard to humans based on mammalian carcinogenicity studies, mode of action data and epidemiology literature.

While there may be some limited evidence of carcinogenicity in the rat study, the liver tumours have been demonstrated to be non-relevant to humans via a mode of action study. Additionally, no tumours were observed in the mouse study, which is acceptable for risk assessment purposes. The epidemiology literature data suggests there is no clear evidence that (S)-metolachlor poses a carcinogenic hazard to humans.

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Appendix 1: Epidemiology Literature Review - J Tomenson, Causation Ltd, November 2021.

Reviews of key studies and assessment of epidemiological evidence of carcinogenicity

Alavanja et al (2004)

The authors examined the relation between 50 agricultural pesticides and lung cancer incidence in the Agricultural Health Study. Follow up was from enrolment (1993–1997) to end 2001. One lung cancer case diagnosed after enrolment was excluded from analyses because an earlier diagnosis of lung cancer was made prior to enrolment. The authors reported lung cancer risk among the 57,284 applicators for the seven pesticides including metolachlor which showed some evidence of an exposure-response relation with lifetime exposure days. Overall, applicators experienced a significantly lower risk of lung cancer compared with the general population (standardised incidence ratio = 0.44; 95% CI, 0.39 - 0.49). The logistic regression analyses used two referent groups (non-exposed or applicators in the lowest tertile of specific pesticide use) and adjusted for smoking, age, gender, and total days of any pesticide application. For the highest category of lifetime exposure days to metolachlor (> upper sextile=457), significantly elevated Odds Ratios (OR) of 4.1 (95% CI, 1.6 – 10.4) and 5.0 (95% CI, 1.7 – 14.9) were reported for the non-exposed and low-exposed referent groups, respectively. Positive trends were seen for both referent group analyses. In addition, analyses were performed using intensity-weighted days of pesticide exposure, but the trend with exposure was no longer significant (p=0.67) and the OR for the highest exposure group reduced to 2.3, (95% CI: 0.9 - 5.5) compared to the non-exposed (low-exposed referent results not reported). It was noted that the intensity-weighted exposure metric gives particular weight to dermal exposure and not to potentially more relevant respiratory exposure.

The study has a number of limitations. No attempt was made to adjust for correlated pesticides and only 150 (96 unexposed, 54 exposed) of the 240 lung cancers observed among applicators could be included in the exposure response analyses because of incomplete information. It also seems implausible that 46 (19%) lung cancer cases did not have smoking information compared to 6% of noncases. The results cannot be regarded as independent of those reported by Rusiecki et al (2006) which added an extra year of follow up, and the lung cancer findings of both Alavanja et al (2004) and Rusiecki et al (2006) are clearly superseded by the study of Silver et al (2015) which had a much longer follow up period, and included a minimum of 510 (330 unexposed, 180 exposed) lung cancer cases in exposure response analyses. Both of the later studies are methodologically superior to Alavanja et al (2004), and both adjusted for exposures to correlated pesticides while Silver et al (2015) also selected a 5-year lag for the primary analyses, discounting the five most recent years of exposure which are unlikely to be relevant for lung cancer.

Rusiecki et al. (2006)

The authors examined cancer incidence among pesticide applicators exposed to metolachlor in the Agricultural Health Study. Follow up was extended by an extra year to the earlier study by Alavanja et al. (2004), but 6,043 applicators who did not provide information on metolachlor use and 1,075 with any cancer diagnosis before enrolment were excluded. Among 50,193 subjects with complete exposure information for metolachlor, 47% reported ever having applied or mixed metolachlor. Poisson regression analyses for individual cancer sites were used to estimate rate ratios (RR) associated with tertiles of lifetime exposure days or intensity-weighted exposure days using two referent groups (non-exposed or lowest tertile of metolachlor use). Another refinement was the adjustment for exposure to 5 pesticides whose use was most highly correlated with metolachlor. Results were only reported for cancer sites with more than 20 exposed cases and more than 5 cases

in each exposure category (all cancers combined, oral cavity, prostate cancer, lung cancer, colon cancer, all lymphohematopoetic cancers and non-Hodgkin lymphoma). In addition, the authors determined that the low-metolachlor exposed applicators were more similar to the applicators in the 2 highest tertiles than the non-metolachlor exposed applicators, and only results for this referent group were presented. For lung cancer (46 exposed cases), there was a nonsignificant, increased risk for applicators in the highest category of lifetime exposure days to metolachlor (> upper sextile=116) (RR = 2.37; 95%CI, 0.97–5.82), and the test for trend was significant (p-trend = 0.03), but there was no association for intensity-weighted lifetime days. Residual confounding from smoking could not be ruled out. For prostate cancer there was some evidence of decreased risk in the highest tertile of exposure for both exposure metrics, but trend tests were not significant. The authors concluded that no clear risk for any cancer subtype was found for exposure to metolachlor. Not surprisingly an association between lung cancer and metolachlor was observed given the large overlap with Alavanja et al (2004), but a lower increased risk was reported for the highest exposure group. However, the sextile of lifetime exposure days (116) in the current study is lower than that reported by Alavanja et al (2004) (457 days) and is equal to the highest tertile of lifetime exposure days reported by that study. However, the lung cancer results are clearly superseded by the study of Silver et al (2015) which had a much longer follow up period, and included a minimum of 510 (330 unexposed, 180 exposed) lung cancer cases in exposure response analyses and selected a 5-year lag for the primary analyses, discounting the five most recent years of exposure which are unlikely to be relevant for lung cancer.

Silver et al. (2015)

The authors updated the study by Rusiecki et al (2006) by extending follow-up of the cohort (through 2010 in North Carolina and 2011 in Iowa) and incorporating new exposure information about pesticide use during the most recent year of application from a follow-up interview conducted approximately 5 years after enrolment (1999–2005) and completed by 63% of applicators. Analyses included a data-driven multiple imputation for days of use for applicators who did not complete the 1999–2005 questionnaire. Of the 49,616 eligible applicators with sufficient information to quantify days of metolachlor use and no cancer diagnosis (other than nonmelanoma skin cancer) before enrolment, 53% were determined to have ever used metolachlor. Poisson regression was used to evaluate relations between cancer incidence and two metrics of metolachlor use (lifetime days, intensity-weighted lifetime days). As noted by the earlier investigation (Rusiecki et al, 2006), the demographic characteristics for groups with higher metolachlor use were more similar to those using less metolachlor than to unexposed applicators. Two sets of analyses were performed: one restricted to person-time after first metolachlor use (person-time in the low metolachlor use category as referent) and a second without this restriction (unexposed person-time as the referent). Primary analyses incorporated 5-year lag (i.e. discounting the five most recent years of exposure) to account for disease latency, but unlagged analyses were also performed. Adjustment was also made for the five most highly correlated pesticides (not the same 5 as in the earlier investigation). Rate ratios (RR) were reported for all cancers with 20 or more exposed cases by quartiles of lifetime days and intensity-weighted lifetime days of metolachlor use (quartiles based on the distribution among exposed cancer cases).

For all cancers combined and 22 cancer subtypes, there were no significant trends for both lifetime and intensity-weighted lifetime days of metolachor use in analyses restricted to person-time after first metolachlor use with applicators in the lowest quartile of exposure as referent group, and no significantly increased risks for any exposure quartile. Analyses with the unexposed as the referent did not change the conclusions for most outcomes, but statistically significant positive trends with lifetime days and intensity-weighted lifetime days of exposure to metolachlor were reported for liver

cancer and follicular cell lymphoma. Stronger associations with lifetime-days were observed for both liver cancer (p-trend<0.01) and follicular cell lymphoma (p-trend=0.03), and for liver cancer, statistically significant increases in liver cancer risk were observed in the upper third (RR = 3.06; 95 % CI, 1.05 – 8.9) and fourth quartiles (RR = 3.99; 95 % CI, 1.43 – 11.1), and a statistically significant increase in follicular cell lymphoma risk in the fourth quartile (RR = 2.89; 95 % CI, 1.13 – 7.38). Weaker associations were observed with intensity-weighted lifetime days of exposure: liver cancer (p-trend=0.03) and follicular cell lymphoma (p-trend=0.04). The suggestion of increased lung cancer risk at high levels of metolachlor use in this cohort (Alavanja et al, 2004; Rusiecki et al, 2006) was not confirmed, and an inverse relation was observed with reduced risk for applicators with the highest quartile of lifetime days exposure (RR= 0.70; 95% CI, 0.47 – 1.02) with the unexposed as the referent.

The only evidence of a trend with exposure and increased risk for liver and follicular cell cancer was seen in analyses using the unexposed as the referent. However, both Silver et al (2015) and Rusiecki et al (2006) observed that demographic characteristics for groups with higher metolachlor use were more similar to those using less metolachlor than to unexposed applicators. For this reason, Rusiecki et al (2006) argued that the low exposure group was more appropriate as a reference group for the Poisson regression analyses than the unexposed group because difference with respect to baseline characteristics might introduce residual confounding from a variety of unidentified sources, and Rusiecki et al (2006) only reported findings for analyses using the low exposure group as the referent.

Barry et al (2011), Koutros et al (2010)

Barry et al (2011) and Koutros et al (2010, 11) are based on the same nested case control study of prostate cancer designed to study gene-pesticide interactions. The incident cases of prostate cancer (n=776) and frequency matched 2:1 controls (n = 1444) were nested in the AHS cohort. Barry et al (2011) reported a just significant decreasing trend (p-trend=0.02) with metolachlor exposure, and an OR of 0.77 (95 % CI, 0.6 – 0.99) for applicators with high exposure (intensity-weighted lifetime days > median) compared to unexposed applicators. However, the analysis was only adjusted for age and state, and based on 212 exposed cases. In contrast, the prospective study of the same AHS cohort by Silver et al (2015) with longer follow up included approximately 5 times as many exposed prostate cancer cases and was able to adjust for other factors including correlated pesticides, but did not observe a significant trend with intensity-weighted lifetime days or lifetime days of metolachlor exposure in analyses with both unexposed or low exposed referent groups, and prostate cancer risk was not significantly reduced among workers in the highest quartile of intensity-weighted lifetime days compared to unexposed applicators (OR=0.92; 95% CI 0.78–1.08). Barry et al (2011) reported no significant interactions between metolachlor and any of the haplotypes investigated in this study.

Koutros et al (2010) evaluated the interaction among pesticide use, genetic variation on chromosome 8q24, and risk of prostate cancer among the same 2220 AHS nested case control subjects. Fifteen of 211 8q24 variants were examined for potential effect modification by use of 49 specific pesticides. These included all variants associated with prostate cancer at the P < 0.01 level and 3 previously identified variants. The association between each 8q24 variant and prostate cancer was examined for 3 exposure strata; unexposed, low exposure (< median lifetime days) and high exposure (≥ median lifetime days). Of the 735 interactions examined, the authors highlighted 24 that had a P for interaction <0.20 (based on pesticide defined as ever/never) and which showed an increasing trend in risk across strata. Apart from two variant interactions with fonofos, none of the 24 interactions remained significant after P values were corrected to take account of the large number of tests performed, including a borderline significant interaction (uncorrected P = 0.05) between metolachlor

and rs12547643, a variant that had not been previously identified as associated with prostate cancer. There was no a priori reason to expect effect modification by metolachlor as prostate cancer risk decreased with increasing metolachlor exposure, and a previous AHS report provided no evidence that a family history of prostate cancer modified risk among those exposed to metolachlor (Alavanja et al, 2003).

Andreotti et al (2010)

This AHS study examined the association between body mass index (BMI) and the risk of cancer at 17 sites, and the interaction between BMI and pesticide use. Subjects were selected for inclusion on the basis of whether BMI information was available and the study included both applicators and their spouses. BMI information was available for 70.7% of pesticide applicators and 84.8% of spouses. Overall, 76% had a BMI taken from the enrolment questionnaire, but the other subjects had a BMI recorded either 5 years after enrolment or taken from 1985 driver's license data (several years before enrolment). The proportion of applicators with an enrolment BMI was much lower than 76% as only 44% completed the take-home questionnaire used to obtain this information, whereas 81% of spouses had completed the relevant questionnaire. Associations between BMI and cancer were evaluated using Cox Proportional-Hazard regression models. The interaction between pesticide use and BMI on colon cancer risk in men was evaluated for 22 pesticides and any organochlorine or organophosphate insecticide. Among all males, there was a significant linear trend between colon cancer and BMI (Hazard Ratio (HR)=1.05, 95% CI 1.02–1.09, p-trend=0.005), and metolachlor had a statistically significant modifying effect (p=0.02). There was a significant linear trend among male users of metolachlor (HR=1.09, 95% CI 1.04–1.15, p-trend=0.001), but not among non-users (HR=1.01, 95% CI 0.96–1.06, p-trend=0.70) although some relationship between colon cancer and BMI would be expected among non–users based on evidence from other studies. A statistically significant modifying effect was observed for 2 other pesticides (alachlor and carbofuran), but it is noted that 11 pesticides without a significant interaction had significant associations between colon cancer and BMI, but non-users of these pesticides did not.

The study has a number of limitations including the large proportion of applicators excluded because they had no BMI data, and the need to use BMI information collected either several years after or before enrolment for many without BMI data at enrolment. The investigators only adjusted for the 2 other pesticides with a statistically significant modifying effect when exploring possible confounding by use of multiple pesticides, but some of the 11 other pesticides with significant associations between colon cancer and BMI among users may have been more strongly correlated with metolachlor. No sensitivity analyses were performed to assess whether BMI at enrolment gave different findings, and no attempt was made to compare the pesticide use of applicators with and without BMI data. In addition, exposure was only assessed as ever/never use, and it is not possible to assess whether exposure to metolachlor increased the risk of colon cancer either overall, or within different BMI categories. Andreotti et al (2010) concluded that their findings should only be considered hypothesis generating for future studies.

Leon et al (2019)

This study investigated the relationship of ever use of 14 selected pesticide chemical groups and 33 individual active chemical ingredients including metolachlor with non-Hodgkin lymphoid malignancies(NHL) overall or 5 major subtypes including follicular cell lymphoma, in a pooled analysis of three large agricultural worker cohorts: AHS (USA), AGRICAN (France) and CNAP (Norway). However, metolachlor analyses only included subjects from the AHS (n=51,167) and AGRICAN (n=127,282) as propachlor was the only chloroacetanilide herbicide for which exposure

information was available in Norway. Cox regression models were used to estimate cohort specific hazard ratios (HRs) which were combined using random effects meta-analysis to calculate meta-HRs. Exposure was self-reported in the AHS but was derived from self-reported history of crops cultivated combined with crop-exposure matrices in AGRICAN. Approximately half of the AGRICAN cohort (51%) consisted of retired farm owners and farm workers, and 44% of AGRICAN participants were female, compared with only 3% in AHS. In AHS, nearly all cohort members were applicators who used pesticides (99%) used but the percentage of pesticide users was 68% in AGRICAN. Metolachlor was not associated with NHL (HR=0.99, 95% CI 0.84–1.17: 358 exposed) and all major subtypes including follicular cell lymphoma (HR=1.05, 95% CI 0.59–1.86: 43 exposed). There was also no evidence of heterogeneity of effect between the two populations.

Brouwer et al (2017) reported metolachlor exposure prevalence for the same AHS and AGRICAN cohorts studied by Leon et al (2019). These were higher for the AHS cohort (male 56%, female 20%) than AGRICAN (male 37%, female 3%), reflecting the fact that only 68% of the AGRICAN cohort were pesticide users. However, the number reporting exposure to metolachlor in the AHS cohort (n=28,162) was only slightly higher than the number estimated to be exposed in the AGRICAN cohort (n=27,715). Leon et al (2019) didn't report HRs for the association between follicular cell lymphoma and metolachlor separately for the AHS and AGRICAN cohorts, but the overall HR of 1.05 and the lack of evidence of heterogeneity, suggests that the association in the AHS cohort was weaker than reported by Silver et al (2015). Silver et al (2015) did not report the RR for ever exposed to metolachlor versus never exposed, but the RRs for the 4 quantiles were 0.93, 2.43, 1.76 and 2.89, and those for the second and highest quartiles were significantly elevated. However, there are some differences between the AHS cohorts included in the two studies, and the analysis approaches. Both studies used the same period of follow up, but the cohort studied by Silver et al (2015) included fewer subjects (n=49,616). The difference largely resulted because Silver et al (2015) excluded 6,259 subjects who did not provide sufficient information to quantify metolachlor use, whereas the current study excluded 4,916 commercial applicators. The current study included 64 cases of follicular lymphoma from the AHS, and a further 34 AGRICAN cases. Silver et al (2015) included 55 cases (31 exposed to metolachlor), but the current study does not report how many of the 43 exposed cases came from each cohort. Another difference between Silver et al (2015) and the current study is that the former study reported Poisson regression analyses whereas the current study reported Cox regression analyses.

Overall assessment

Lung cancer

Although Alavanja et al (2004) and Rusiecki et al (2006) reported some evidence of excess lung cancer at higher lifetime days of metolachlor exposure, the study of Silver et al (2015) reported no exposure response and no notable or statistically significant increase in any exposure quartile, and a nonsignificant inverse relation was observed with unexposed applicators as the reference group. The earlier associations were most likely due to chance as Silver et al (2015) had a much longer follow up period, and included a minimum of 180 exposed lung cancer cases in exposure response analyses compared to the 54 cases and 46 cases included by Alavanja et al (2004) and Rusiecki et al (2006), respectively. In addition, Silver et al (2015) selected a 5-year lag for the primary analyses, discounting the five most recent years of exposure which are unlikely to be relevant for lung cancer. However, Silver et al (2015) stated that the apparent attenuation of lung cancer risk may indicate that diminishing use has reduced risk, or that latency may be important. Rusiecki et al (2006) reported that 35.5% of higher exposed participants were aged < 40 years and a further 31.1% were aged between 40 and 50 years, hence the increased latency (and additional lung cancer cases) from

incorporating 8-9 years of additional follow up should have given more power to detect a real exposure effect on lung cancer risk. Silver et al (2015) concluded that use of metolachlor in the cohort had diminished because only 15% of applicators reported using metolachlor in the most recent farming year, in contrast with 48% reporting ever-use at enrolment. However, these figures aren't comparable, and even if use had diminished and exposure reduced, the incorporation of additional exposure information for five years since enrolment is likely to have had very little impact on the exposure distribution derived from the enrolment questionnaire (the proportion exposed to metolachlor only increased from 47% to 53% when such information was incorporated). Overall, the AHS studies do not suggest that lung cancer is associated with metolachlor exposure.

Colon cancer

Andreotti et al (2010) reported that there was a significant linear trend between colon cancer and BMI (Hazard Ratio (HR)=1.05, 95% CI 1.02–1.09, p-trend=0.005) among all males, and metolachlor had a statistically significant modifying effect (p=0.02). There was a significant linear trend among male users of metolachlor (HR=1.09, 95% CI 1.04–1.15, p-trend=0.001), but not among non-users (HR=1.01, 95% CI 0.96–1.06, p-trend=0.70), although some relationship between colon cancer and BMI would be expected among non–users based on evidence from other studies. A significant interaction was also reported for 2 other pesticides (alachlor and carbofuran) out of 22 pesticides and any organochlorine or organophosphate insecticide, but it is noted that 11 pesticides without a significant interaction had significant associations between colon cancer and BMI, but non-users of these pesticides did not. The HR was significantly elevated for applicators exposed to metolachlor with BMI > 30, but this would be expected as it was significantly elevated for all male applicators with BMI of 30–34.9 and ≥35 (and applicators exposed to 12 other pesticides and organochlorines and organophosphates). The study has a number of limitations including the large proportion of applicators excluded because they had no BMI data, and the need to use BMI information collected several years after or before enrolment for many without BMI data at enrolment. In addition, the investigators only adjusted for the 2 other pesticides with a statistically significant interaction effect when exploring possible confounding by use of multiple pesticides, but 11 other pesticides demonstrated similar but nonsignificant associations, and may have been strongly correlated with metolachlor. Furthermore the authors were only able to examine exposure split as ever/never and it is not possible to assess whether exposure to metolachlor increased the risk of colon cancer either overall, or within different BMI categories. However, other AHS studies have reported no association between colon cancer and metolachlor (Rusiecki et al, 2006; Lee et al., 2007; Silver et al., 2015), and Silver et al (2015) reported lower colon cancer risk among exposed applicators in all quartiles for both exposure metrics than among unexposed applicators. However, the number of applicators (40,515) included in the study by Andreotti et al (2010) is much lower than most other AHS cancer incidence studies because of the exclusion of applicators with missing BMI data. Given the lack of any evidence of increased colon cancer risk among metolachlor users in other studies, and the lack of a mechanism to explain findings, the authors were right to conclude that their findings should only be considered hypothesis generating for future studies.

Liver cancer

Silver et al (2015) noted that, to their knowledge, their study is the first occupational epidemiology study to report a positive association between liver cancer and metolachlor exposure, although they also describe their findings as "suggestions" of positive associations between metolachlor use and incidence of liver cancer. Liver cancer was of a priori interest because of what they described as mixed findings from rodent studies. Statistically significant findings were only observed in analyses with the unexposed group as referent. However, Silver et al (2015) observed that demographic

characteristics for groups with higher metolachlor use were more similar to those using less metolachlor than to unexposed applicators. Rusiecki et al (2006) also noted that the low-metolachlor exposed applicators were more similar to the higher exposed applicators than the non-metolachlor exposed applicators, and that this made the group more appropriate as a reference group for the Poisson regression analyses because difference with respect to baseline characteristics might introduce residual confounding from a variety of unidentified sources. Rusiecki et al (2006) performed analyses using both the low exposed tertile and the non-exposed as referents, but did not report findings for analyses using the unexposed group as the referent. In addition, the trend observed by Silver et al (2015) in analyses with the unexposed group as referent was weaker for the intensity-weighted lifetime days exposure metric (p-trend=0.03) although this would be expected to be the most relevant exposure metric for a cancer such as liver cancer. Similar AHS investigations of cancer incidence for other pesticides only reported analyses based on cumulative lifetime days as online supplementary information (Lerro et al, 2018; 2020). No evidence of a trend with metolachlor exposure (p=0.44) was observed by Silver et al (2015) in the most appropriate analysis (lowest quartile of exposure as referent group and the intensity-weighted lifetime days exposure metric), and the risk of liver cancer was also not significantly elevated among applicators in the highest quartile of exposure (RR = 1.71; 95% CI, 0.33–8.83). In addition, residual confounding cannot be ruled out. Hepatitis infection was not mentioned, and no adjustment made for body mass index. Alcohol consumption was only assessed in the year before enrolment, and cigarette smoking only categorised into 3 groups on the basis of the pack-years of smokers (never/low/high). In addition there were few cases of liver cancer (23 exposed in the 5-year lag analysis) which restricted the ability of the investigators to look at longer lag periods (Lerro et al, 2020), or interactions between exposure and known risk factors (Lerro et al, 2018). Furthermore, Lerro et al (2019) reported a large deficit excess of liver intrahepatic bile duct cancer among private pesticide applicators in the AHS (SIR = 0.56; 95% CI 0.45, 0.70).

In conclusion, there is only weak evidence of association in the AHS cohort, and no confirmatory evidence from other cohorts.

Follicular cell lymphoma

Silver et al (2015) also found suggestions of a positive association between metolachlor use and incidence of follicular cell lymphoma. In this case there was no a priori reason for focusing on follicular cell lymphoma, and no evidence of associations between metolachlor use and either NHL or lymphohematopoietic cancers in general in both their study or a previous analysis of AHS data (Rusiecki et al, 2006). The findings mirrored those for liver cancer and there was no evidence of a trend with metolachlor exposure (p=0.21) in the most appropriate analysis (lowest quartile of exposure as referent group and intensity-weighted lifetime days exposure metric), and the risk of follicular cell lymphoma was not significantly elevated among applicators in the highest quartile of exposure (RR = 2.08; 95% CI, 0.61–7.10). Leon et al (2019) combined results from the AHS study and the French AGRICAN study. Both cohorts included similar numbers of workers reporting exposure to metolachlor (AHS, n=28,162; AGRICAN, n=27,715), but the AGRICAN cohort contained over 4 times more subjects unexposed to metolachlor. The meta-estimate for follicular cell lymphoma (HR=1.05, 95% CI 0.59–1.86: 43 exposed) does not suggest an association with metolachlor exposure, and there was no evidence of heterogeneity between the AHS and AGRICAN studies.

In conclusion, there is only weak evidence of association from one study of the AHS cohort, and no confirmatory evidence from a later study that combined findings from the AHS and AGRICAN studies.

Prostate cancer

Barry et al (2011) reported a just significant decreasing trend (p-trend=0.02) with metolachlor exposure, and an OR of 0.77 (95 % CI, 0.6 – 0.99) for applicators with high exposure (intensity-weighted lifetime days > median) compared to unexposed applicators. However, the analysis was only adjusted for age and state, and based on 212 exposed cases. In contrast, the prospective study of the same AHS cohort by Silver et al (2015) with longer follow up included approximately 5 times as many exposed prostate cancer cases and was able to adjust for other factors including correlated pesticides. In the most comparable analysis, Silver et al (2015) did not observe a significant trend with intensity-weighted lifetime days (p-trend=0.26) in the analysis with unexposed referent group, and the risk was not significantly reduced among workers in the highest quartile of exposure (OR=0.92; 0.78–1.08). Koutros et al (2010) examined 735 interactions between 15 8q24 region variants associated with increased prostate cancer risk, and 49 specific pesticides including metolachlor. However, apart from two variant interactions with fonofos, none remained significant after P values were corrected to take account of the large number of tests performed, including the borderline significant interaction (uncorrected P = 0.05) between metolachlor and rs12547643, a variant that had not been previously identified as associated with prostate cancer. Koutros et al (2013) also found no evidence that either total or aggressive prostate cancer was associated with metolachlor exposure. There was also no evidence of effect modification from a family history of prostate cancer among individuals with exposure to metolachlor (Alavanja et al, 2003). Overall, there is no persuasive evidence that prostate cancer is associated with metolachlor exposure.

Conclusion

Silver et al (2015) reported some suggestive evidence that liver cancer and follicular cell lymphoma may be associated with metolachlor exposure. However, there were no statistically significant increases in risk for applicators in any quartile of exposure when the analysis was performed using the most appropriate reference group consisting of applicators with the lowest quartile of exposure, and no significant trends with exposure. Furthermore, even in analyses using applicants unexposed to metolachlor as the reference group, trends with exposure were much weaker for the intensity-weighted exposure metric and barely reached statistical significance. For follicular cell lymphoma, additional evidence is available from a combined analysis of the AHS cohort and the much larger French AGRICAN cohort which included similar numbers of users of metolachlor. The combined analysis reported no evidence of an association between follicular cell lymphoma and metolachlor exposure. There is also no supporting evidence of an association between liver cancer and metolachlor from the AHS or other epidemiological studies, and Silver et al (2015) noted that further follow-up is needed to facilitate assessment of whether the differences in the results reflect greater statistical power with a larger reference category or other exposure related factors. It is also noted that further follow-up would permit better assessment of the role of latency in these associations and whether a longer lag period may be more biologically plausible. There is no persuasive evidence that any other cancer subtype (including lung, colon and prostate) is associated with metolachlor exposure.

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