Time of vaginal opening in the study of Atrazine reported by Ashby et al (2002).

Ashby et al (5) have reported a delay in vaginal opening (VO) for Alderley Park (AP) Wistar and Sprague Dawley (SD) rats exposed to the herbicide Atrazine. Laws et al. (8) subsequently confirmed this effect in Wistar rats, and both groups coincide in their conclusion that activity is restrained to doses of Atrazine above 100mg/kg/day. At the December 2003 EDMVS meeting, the age of VO in both control AP Wistar and SD rats in the study of Ashby et al. (5) was compared to the age of VO for Wistar rats in the study by Laws et al. (8), as well as the age of VO in control SD rats in the studies by Lewis et al. (10) and in the unpublished studies of Rocca and Patel (30) and George et al (.9). The mean age of VO in the control AP Wistar and SD rats (5) were between 5 to 7 days later than for these other studies. This reply is intended to put these findings into a broader perspective.

The data shown in Figure 1 demonstrate that the mean day of VO in our Wistar rat study of Atrazine was comparatively late when compared to our other research data. Also, VO occurred at the upper end of the regulatory database available for these same rats within Central Toxicology Laboratory (CTL) of Syngenta Crop Protection Inc. The lower panel of Figure 1 shows a limited (but unbiased) search of the recent literature, and includes the results from 3 studies on Atrazine reported by Laws (8). The shaded entries in Figure 1 are for SD rats.

The value of 39 we reported for VO in SD rats in the Atrazine study (5) coincides exactly with that found in our earlier study of BPA (4). At the time of that earlier study we were concerned at the comparative lateness of VO in SD rats, but this was the first time we had used them for such purposes – and the value of 39, although high, was lower than that reported for SD rats by Chahoud and his colleagues (15); that being the initial BPA study that we were attempting to repeat in our BPA study (4).

We have no explanation for our relatively late VO in these two studies (4,5). However, we were not concerned because measurement of VO is generally agreed to be a relative assessment, i.e., test groups are compared to the concurrent control group. One variable affecting the mean day of VO is the diet used (2), but that could not be a factor in this case because our usual RM1 diet was employed for all of our research studies cited. Further, variability of up to 2 days may occur between laboratories dependent upon whether the onset of completion of VO is monitored. However, although that is often not made clear in publications, both Laws et al (8) and Ashby et al (5) recorded the completion of VO.

Rivest (31) has discussed this subject in detail in 1991 , and concluded as follows - ‘In summary, there is more need to understand the role of the environment, including that of the mother early in fetal and neonatal life. It is suggested that the mechanisms underlying organ growth are set for a given species, while developmental and environmental factors fix the timing of vaginal opening and first ovulation. In the rat, there appear to be two times which are preferred for vaginal opening, given the laboratory conditions that have been used in the last 20 or so years: an early period, at 31-35 days, and a late period, at 36-40 days. An explanation for this dichotomy would be that a combination of parameters (not necessarily always the same) is needed for vaginal opening’.

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January 13 2004

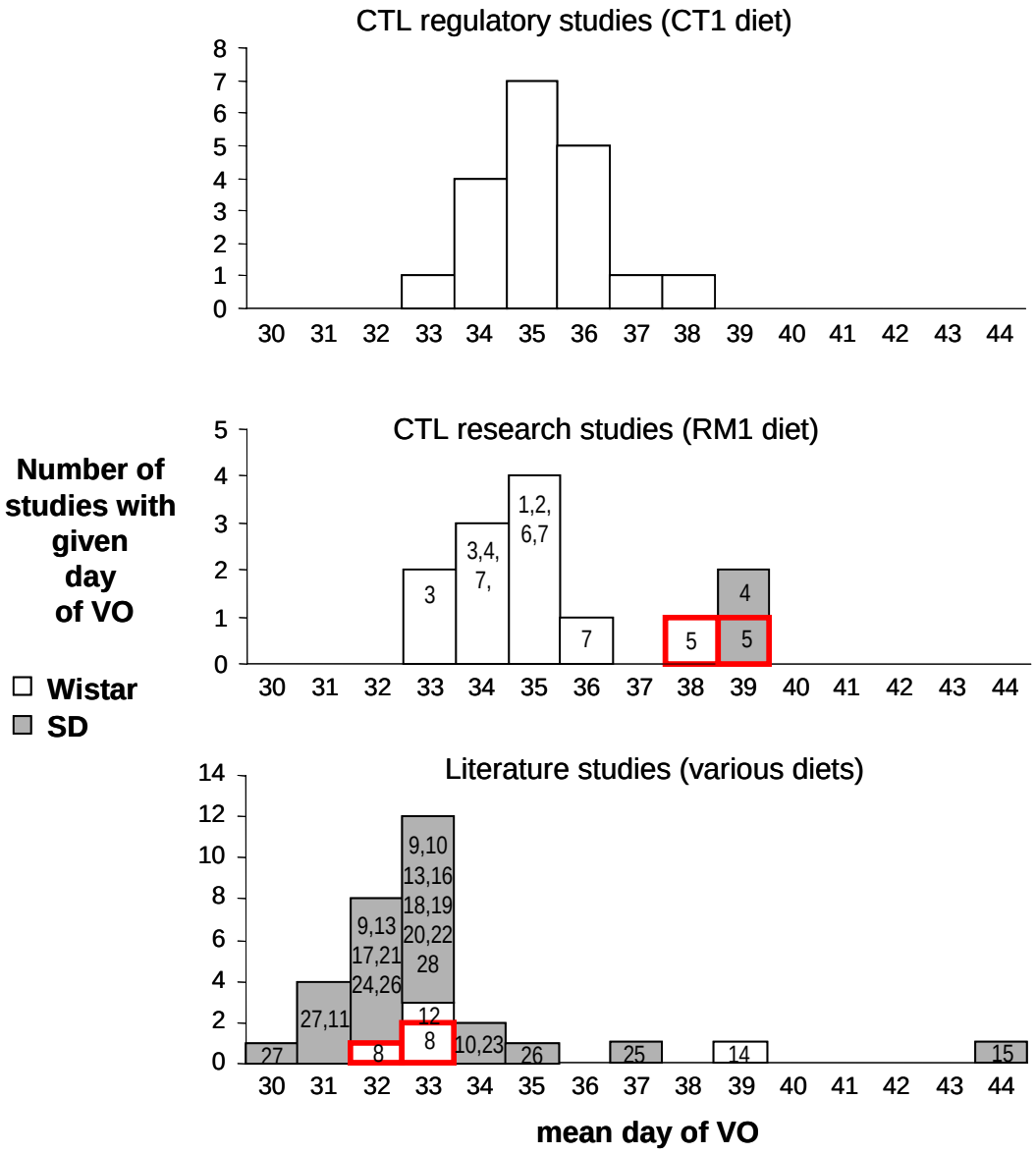

Figure 1. Mean day of VO for control rats as reported in studies from 1997-2003. The height of the bars indicates the number of studies with that day of VO for control groups (abscissa). The numbers within the bars refer to the reference number of the studies considered. Some references contain multiple studies. The studies on Atrazine from Ashby et al (5) and Laws et al (8) are outlined in red. The regulatory studies are from CTL developmental and multigenerational studies conducted from 1997-2003 (unpublished). The research studies are as published by Ashby et al. from 1997-2003. Also shown are some readily available literature citations also from 1997-2003.

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